TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

ENDOMET DATABASE –
A means to identify novel diagnostic and prognostic tools for endometriosis

Michael Gabriel

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1597 | MEDICA – ODONTOLOGICA | TURKU 2021

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

ENDOMET DATABASE –

A means to identify novel diagnostic and prognostic tools for endometriosis

Michael Gabriel

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1597 | MEDICA – ODONTOLOGICA | TURKU 2021

University of Turku

Faculty of Medicine
Institute of Clinical Medicine
Department of Obstetrics and Gynaecology
Institute of Biomedicine, Research Centre for Integrative Physiology and Pharmacology
Doctoral programme in Clinical Research

Supervised by

Adjunct Professor Antti Perheentupa
Faculty of Medicine
Department of Obstetrics and Gynaecology
Institute of Biomedicine
Research Centre for Integrative Physiology and Pharmacology
University of Turku
Turku, Finland

Professor Matti Poutanen
Faculty of Medicine
Institute of Biomedicine
Research Centre for Integrative Physiology and Pharmacology
University of Turku
Turku, Finland

Reviewed by

Adjunct Professor Hanna Savolainen-Peltonen
Faculty of Medicine
Department of Obstetrics and Gynecology
University of Helsinki
Helsinki, Finland

Professor Matti Nykter
Faculty of Medicine and Health Technology
Tampere University
Tampere, Finland

Opponent

Professor Johanna Arola
Faculty of Medicine
Department of Pathology
University of Helsinki
Helsinki, Finland

The originality of this publication has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-8712-2 (PRINT)
ISBN 978-951-29-8713-9 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama, Turku, Finland 2021

Dedicated to my loving family and many friends

UNIVERSITY OF TURKU
Faculty of Medicine
Institute of Clinical Medicine
Department of Obstetrics and Gynaecology
Institute of Biomedicine
Research Center for Integrative Physiology and Pharmacology
Michael Gabriel: ENDOMET database – A means to identify novel diagnostic and prognostic tools for endometriosis
Doctoral Dissertation, 267 pp.
Doctoral Programme in Clinical Research (DPCR)
November 2021

ABSTRACT

Endometriosis is a common benign hormone reliant inflammatory gynecological disease that affects fertile aged women and has a considerable economic impact on healthcare systems. Symptoms include intense menstrual pain, persistent pelvic pain, and infertility. It is defined by the existence of endometrium-like tissue developing in ectopic locations outside the uterine cavity and inflammation in the peritoneal cavity. Endometriosis presents with multifactorial etiology, and despite extensive research the etiology is still poorly understood. Diagnostic delay from the onset of the disease to when a conclusive diagnosis is reached is between 7–12 years. There is no known cure, although symptoms can be improved with hormonal medications (which often have multiple side effects and prevent pregnancy), or through surgery which carries its own risk. Current non-invasive tools for diagnosis are not sufficiently dependable, and a definite diagnosis is achieved through laparoscopy or laparotomy.

This study was based on two prospective cohorts: The ENDOMET study, including 137 endometriosis patients scheduled for surgery and 62 healthy women, and PROENDO that included 138 endometriosis patients and 33 healthy women.

Our long-term goal with the current study was to support the discovery of innovative new tools for efficient diagnosis of endometriosis as well as tools to further understand the etiology and pathogenesis of the disease. We set about achieving this goal by creating a database, EndometDB, based on a relational data model, implemented with PostgreSQL programming language. The database allows e.g., for the exploration of global genome-wide expression patterns in the peritoneum, endometrium, and in endometriosis lesions of endometriosis patients as well as in the peritoneum and endometrium of healthy control women of reproductive age. The data collected in the EndometDB was also used for the development and validation of a symptom and biomarker-based predictive model designed for risk evaluation and early prediction of endometriosis without invasive diagnostic methods. Using the data in the EndometDB we discovered that compared with the eutopic endometrium, the WNT- signaling pathway is one of the molecular pathways that undergo strong changes in endometriosis. We then evaluated the potential role for secreted frizzled-related protein 2 (SFRP-2, a WNT-signaling pathway modulator), in improving endometriosis lesion border detection. The SFRP-2 expression visualizes the lesion better than previously used markers and can be used to better define lesion size and that the surgical excision of the lesions is complete.

KEYWORDS: endometriosis, database, early diagnosis, predictive model, diagnostic delays, risk assessment, relation data model, PostgreSQL, WNT signaling, secreted frizzled-related protein 2, lesion border.

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen laitos
Synnytys- ja naistentautioppi
Biolääketieteen laitos
Integratiivisen fysiologian ja farmakologian tutkimuskeskus
Michael Gabriel: ENDOMET tietokanta – Keino tunnistaa uusi diagnostinen ja ennustava työkalu endometrioosille
Väitöskirja, 267 s.
Turun kliininen tohtoriohjelma (TKT)
Marraskuu 2021

TIIVISTELMÄ

Endometrioosi on yleinen hyvänlaatuinen, hormoneista riippuvainen tulehduksellinen lisääntymisikäisten naisten gynekologinen sairaus, joka kuormittaa terveydenhuoltojärjestelmää merkittävästi. Endometrioositaudin oireita ovat mm. voimakas kuukautiskipu, jatkuva lantion alueen kipu ja hedelmättömyys. Sairaus määritellään kohdun limakalvon kaltaisen kudoksen esiintymisenä kohdun ulkopuolella sekä siihen liittyvänä vatsakalvon tulehduksena. Endometrioosin etiologia on monitahoinen, ja laajasta tutkimuksesta huolimatta edelleen huonosti tunnettu. Kesto taudin puhkeamisesta lopullisen diagnoosin saamiseen on usein jopa 7–12 vuotta. Sairauteen ei tunneta parannuskeinoa, mutta oireita voidaan lievittää esimerkiksi hormonaalisilla lääkkeillä (joilla on usein monia sivuvaikutuksia ja jotka estävät raskauden) tai leikkauksella, johon liittyy omat tunnetut riskit. Nykyiset ei-invasiiviset diagnoosityökalut eivät ole riittävän luotettavia sairauden tunnistamiseen, ja varma endometrioosin diagnoosi saavutetaan laparoskopian tai laparotomian avulla.

Tämä tutkimus perustui kahteen prospektiiviseen kohorttiin: ENDOMET-tutkimukseen, johon osallistui 137 endometrioosipotilasta ja 62 terveellistä naista, sekä PROENDO-tutkimukseen, johon osallistui 138 endometrioosipotilasta ja 33 terveellistä naista.

Tässä tutkimuksessa pitkän aikavälin tavoitteemme oli löytää uusia työkalujen endometrioosin diagnosointiin, sekä ymmärtää endometrioosin etiologiaa ja patogeneesiä. Ensimmäisessä vaiheessa loimme EndometDB –tietokannan PostgreSQL-ohjelmointikielellä. Tämän osittain avoimeen käyttöön vapautetun tietokannan avulla voidaan tutkia genomin, esimerkiksi kaikkien tunnettujen geenien ilmentymistä peritoneumissa, endometriumissa ja endometrioosipotilaiden endometrioosileesioissa EndometDB-tietokantaan kerättyjä tietoja käytettiin oireiden ja biomarkkeripohjaisen ennustemallin kehittämiseen ja validointiin. Malli tuottaa riskinarvioinnin endometrioositaudin varhaiseen ennustamiseen ilman laparoskopiaa. Käyttäen EndometDB-tietokannan tietoja havaitsimme, että endometrioositautikudoksessa tapahtui voimakkaita geeni-ilmentymisen muutoksia erityisesti geeneissä, jotka liittyvät WNT-signalointireitin säätelyyn. Keskeisin löydös oli, että SFRP-2 proteiinin ilmentyminen oli huomattavasti koholla endometrioosikudoksessa ja SFRP-2 proteiinin immunohistokemiallinen värjäys erottaa endometrioosin tautikudoksen terveestä kudoksesta aiempia merkkiaineita paremmin. Löydetyllä menetelmällä voidaan siten selvittää tautikudoksen laajuus ja tarvittaessa osoittaa, että leikkauksella on kyetty poistamaan koko sairas kudos.

AVAINSANAT: endometrioosi, tietokanta, varhainen diagnoosi, ennustava malli, diagnostiset viiveet, riskinarviointi, suhdetietomalli, PostgreSQL, WNT-signalointi, erittyvä pörröinen proteiini 2, vaurion raja.

Table of Contents

Abbreviations

AFS	The American Fertility Society
AGI	Artificial General Intelligence
AI	Artificial Intelligence
ANN(s)	Artificial neural network(s)
ANOVA	Analysis of variance
API(s)	Application programming interface(s)
AUC	Area under the curve
BLAST	Basic local alignment search tool
BMI	Body mass index
BMPR1B	Bone Morphogenetic Protein Receptor Type 1B
BSA-PBS	Bovine Serum Albumin in PBS
CA-125 (15-3) (19-9) (72-4)	Cancer antigen 125 (15-3) (19-9) (72-4)
CALD-1	Caldesmon 1
CD10	CALLA, common acute lymphoblastic leukemia antigen, neutral endopeptidase-24.11, EC 3.4.24.11, NEP, encephalokinase, neprilysin
CDCA2	Cell Division Cycle Associated 2
cDNA	Complementary DNA
CE	Controls endometrium
CGRP	Calcitonin gene-related peptide
ChIP-Seq	Chromatin Immunoprecipitation Sequencing
Chr(X)	Chromosome (X-chromosomes)
CI	Confidence interval
CLDN1	Claudin 1
CMH	Sphingolipid monohexosylceramides
CNN	Convolutional neural network
COCP(s)	Combined oral contraceptives pill(s)
COX (-1) (-2)	Cyclooxygenase (1) (2)
CP	Control peritoneum
cRNA	Complementary RNA
CRP	C-reactive protein

CT	Computer tomography
CTNNB1	Catenin Beta 1
CV	Cross Validation
CYP19A1	Cytochrome P450 Family 19 Subfamily A Member 1
DAB	3,3'-Diaminobenzidine
DAPI	4′,6-diamidino-2-phenylindole
DiE	Deep infiltrating endometriosis
DiEB	Deep endometriotic lesions in the bladder
DiEIn	Intestinal endometriotic lesions
DKK1	Dickkopf WNT Signaling Pathway Inhibitor 1
DKK3	Dickkopf WNT Signaling Pathway Inhibitor 3
DMEM/F12	Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12
DUSP22	Dual Specificity Phosphatase 22
E	Endometrium
E1	Estrone
E2	Estradiol
EDTA	Ethylenediaminetetraacetic acid
EF	Endometrial fluid
EFI	Endometriosis Fertility Index
EGF	Epidermal Growth Factor
EHP-30	Endometriosis Health Profile-30
ELISA	Enzyme-linked immunosorbent assay
EMILIN-1	Elastin microfibril interfacer 1
ENDO	Endometriosis: Natural History, Diagnosis, and Outcomes Study
ENDOMET	Novel diagnostic tools for endometriosis and their exploitations for prognosis and prevention of complications project
EndometDB	Turku Endometriosis Database
EO	Extra-ovarian endometriosis
ER (-α) (-β)	Estrogen Receptors (Alpha) (Beta)
ESHRE	European Society of Human Reproduction and Embryology
ESI-FIA-MS/MS	Electrospray ionization flow injection analysis tandem mass spectrometry
ESR2	Estrogen Receptor 2
FA	Adenomyosis
FB	Bladder
FC	Fold change

FDA	Food and Drug Administration
FI	Bowel disease cranial to the rectosigmoid junction
FO	Abdominal wall endometriosis
FRZB	Frizzled Related Protein
FSH	Follicle stimulating hormone
FU	Intrinsic involvement of the ureter
FZD (7) (10)	Frizzled Class Receptor (7) (10)
ggplot2	Data visualization package for the statistical programming language R
GI	Gastrointestinal
GnRH(s)	Gonadotropin releasing hormone(s)
GnRH-a	Gonadotropin releasing hormone-agonist
GREB1	Gremlin 1, DAN Family BMP Antagonist
GUI	Graphical user interface
GWAS	Genome wide association study
HE4	Human epididymal secretory protein E4
HPRT1	Hypoxanthine Phosphoribosyl transferase 1
HR	Hazard ratio
HRT	Hormone replacement therapy
HSD17B6	Hydroxysteroid 17-Beta Dehydrogenase 6
HTML / HTML5	HyperText Markup Language
ID(s)	Identification
ID2	Inhibitor of DNA Binding 2
ID4	Inhibitor of DNA Binding 4, HLH Protein
IFN-γ	Interferon-gamma
IGSF21	Immunoglobin Superfamily Member 21
IHC	Immunohistochemical analysis
IL	Interleukin
IL-12B	Interleukin 12B
IL-12p40	Interleukin 12 subunit p40
IL-12p70	Interleukin 12 composed of p40 and p35 subunits
IL-1R2	Interleukin 1 Receptor Type 2
IL-1Ra	Interleukin 1 Receptor Antagonist
IL-1α	Interleukin 1alpha
IL-1β	Interleukin 1 beta
IP-10	IFN-γ-induced protein-10
IUD	Intrauterine device
IVF	In vitro fertilization
JMP®	Statistical analysis software from SAS Institute
jQuery	JavaScript library

JSON	JavaScript Object Notation
JUN	Jun Proto-Oncogene, AP-1 Transcription Factor Subunit
k	Kappa
K4001	Dako EnVision®+ System-HRP labeled polymer against mouse IgG
K4003	Dako EnVision®+ System-HRP labeled polymer against rabbit IgG
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC/ESI-MS/MS	Liquid Chromatography-Electrospray Ionization-Mass Spectrometry
LC-MS	Liquid chromatography–mass spectrometry
LC–MS/MS	Liquid chromatography–tandem mass spectrometry
LFDA	Local Fisher Discriminant Analysis
LH	Luteinizing hormone
LNG-IUD(S)	Levonorgestrel-releasing intra-uterine device (system)
MALDI-TOF-MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MAPK	Mitogen-Activated Protein Kinase
MDK	Midkine
MDS	Multidimensional scaling
MGMT	O-6-Methylguanine-DNA Methyltransferase
miRNA	microRNA
ML	Machine learning
MME	Membrane Metallo endopeptidase
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
MUC16	Mucin 16, Cell Surface Associated
MYCIN	An early expert system, or AI program, for treating blood infections
NA	Not applicable
NCBI	National Center for Biotechnology Information
ncRNA	Non-coding RNA
NF	Neurofilament
ng	Nanogram
NGS	Next Generation Sequencing
NK	Natural killer
NPV	Negative predictive value
NPY	Neuropeptide Y

NS	Not significant
NSAID(s)	non-steroidal anti-inflammatory drug(s)
OMA	Ovarian cysts or endometrioma
OR	Odds ratio
ORCA	Open-source Report Creator App R Package
ORDBMS	Open-source object-relational database management system
P	P-value
PAPNET	Cytological Screening System
PBS	Phosphate-buffered saline
PBS-T/PBS-Tween	Phosphate-buffered saline solution with Tween® 20
PC.ae.C38.0	1-organyl-2-acyl-sn-glycero-3-phosphocholine
PC.ae.C38.1	1-octadecyl-2-(9Z-eicosenoyl)-sn-glycero-3-phosphocholine
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PDF	Portable Document Format
PE	Patient endometrium
PeLB	Black peritoneal endometriotic lesion
PeLR	Red peritoneal endometriotic lesion
PeLW	White peritoneal endometriotic lesion
PF	Peritoneal fluid
PG	Prostaglandin
PGE2	Prostaglandin E2
PGE2-d4	Prostaglandin E2-d4
PGF2α	Prostaglandin F2 alpha
PGH2	Prostaglandin H2
PGP 9.5	Protein Gene Product 9.5
PGR	Progesterone receptor
PHP	Hypertext Preprocessor
PI3K	Phosphoinositide 3-Kinase
Plotly	Open-source interactive, scientific data visualization
Plotly.js	Plotly JavaScript Open-Source Graphing Library
PostgreSQL	Open-source RDBMS
PP	Patient peritoneum
PPV	Positive predictive value
PR	Pregnancy rate
PRB/ PR-B	Progesterone receptor B
PRE(s)	Progesterone response element(s)

PROENDO	Novel diagnostic tools for endometriosis and their exploitations for prognosis and prevention of complications Cohort II
PVDF	Polyvinylidene fluoride
PWA	Progressive web application
QoL	Quality of life
qRT-PCR	Quantitative reverse transcription PCR
R	Programming language and free software environment for statistical computing and graphics
RA	Risk allele
Ra	Rheumatoid arthritis
RAF	Rapidly Accelerated Fibrosarcoma
RAF_{EUR}	Average risk allele frequency in European samples
RAF_{JPT}	Average risk allele frequency in Japanese samples
rAFS score	Revised American Fertility Society score
RAS	Rat Sarcoma
rASRM	Revised American Society for Reproductive Medicine
RBBP7	RB Binding Protein 7, Chromatin Remodeling Factor
rcorr	Matrix of Correlations and P-values function in R
RDBMS	Relational database management system
RefSeq	NCBI Reference Sequence Database
REV	Deep rectovaginal
RF	Random Forest
RM-one-way ANOVA	One-way repeated measures ANOVA
RNA-Seq	RNA sequencing
ROC	Receiver operating characteristic
SD	Standard Deviation
SDS	Sodium dodecyl sulfate
SERM(s)	Selective estrogen modulator(s)
SF1	Steroidogenic factor 1
SFRP 1	Secreted Frizzled Related Protein 1
SFRP 2	Secreted Frizzled Related Protein 2
siRNA(s)	Small interfering RNA(s)
SMOH	Hydroxysphingomyelins
SNP(s)	Single nucleotide polymorphism(s)
SP	Substance P
SPRM(s)	Selective progesterone modulator(s)
SQL	Structured Query Language
SuL	Sacrouterine ligament lesion
SVG	Scalable Vector Graphics

T	Testosterone
T1-WI / T2-WI	T1-weighted images / T2-weighted images
T-cell	T lymphocyte
TGF-α	Transforming growth factor alpha
TGFβ1	Transforming growth factor beta 1
TGX	Tris-Glycine eXtended
TNFRSF1B	Tumor Necrosis Factor Receptor Superfamily Member 1B
TNF-α	Tumor Necrosis Factor alpha
TP73	Tumor Protein P73
Tris (-EDTA)	tris(hydroxymethyl)aminomethane (-EDTA)
TRIsure	A reagent for fast, simple, scalable purification of high-quality total RNA, or the simultaneous isolation of RNA, genomic DNA, and protein from a wide variety of biological samples.
TSA™	Tyramide signal amplification system detection kits
TVS	Transvaginal sonography
TVUS	Transvaginal ultrasonography
UI	User interface
URL	Uniform Resource Locator
USL	Uterosacral ligaments
VAS	Visual analogue scale
VEGF	Vascular Endothelial Growth Factor
VEGFB	Vascular Endothelial Growth Factor B
VIP	Vasoactive Intestinal Polypeptide
Web UI	Web User Interface
WERF	World Endometriosis Research Foundation
WERF-WHSS World	Endometriosis Research Foundation-Women's Health Symptom Survey
WES	Whole exome sequencing
WFDC2	WAP four-disulphide core domain protein 2
WGS	NGS for whole genome
WISP2	WNT1 Inducible Signaling Pathway Protein 2
WNT	Wingless-Type MMTV Integration Site Family
WNT5A	Wingless-Type MMTV Integration Site Family, Member 5A
Xist	X-inactive specific transcript
ZNF681	Zinc Finger Protein 681
β-catenin	Catenin beta-1

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I **M. Gabriel**, V. Fey, T. Heinosalo, P. Adhikari, K. Rytkönen, T. Komulainen, K. Huhtinen, T D. Laajala, H. Siitari, A. Virkki, P. Suvitie, H. Kujari, T. Aittokallio, A. Perheentupa, M. Poutanen. A relational database to identify differentially expressed genes in the endometrium and endometriosis lesions. *Sci Data*, 2020; 7: 284.

II T. Heinosalo*****, **M. Gabriel***, L Kallio, P. Adhikari, K. Huhtinen, T D. Laajala, E Kaikkonen, A. Mehmood, P. Suvitie, H. Kujari, T. Aittokallio, A. Perheentupa, M. Poutanen. Secreted frizzled-related protein2 (SFRP2) expression promotes lesion proliferation via canonical WNT signaling and indicates lesion borders in extraovarian endometriosis. *Human Reproduction*, 2018; 5: 817–831.

III **M. Gabriel**, P. Adhikari, V. Fey, T D. Laajala, T. Komulainen, T. Heinosalo, K. Huhtinen, P. Suvitie, H. Siitari, H. Kujari, C. Edgren, A. Perheentupa, M. Poutanen. Risk assessments of endometriosis using predictive models with clinical symptoms and serum biomarkers. *Submitted for publication*.

***** Equal contribution as first authors

1 Introduction

Endometriosis is a relatively common chronic and benign estrogen dependent gynecological condition where the endometrial tissues lining the uterus are found outside the endometrial cavity and uterine musculature where it stimulates an inflammatory reaction [1–3]. It is found primarily on the ovaries, the pelvic peritoneum, the bladder, the bowel and in the rectovaginal septum. It typically affects fertile aged women, with a projected prevalence between 1 and 10% estimated to be around 176 million women worldwide. Endometriosis has been linked to severe menstrual pain, infertility and chronic pelvic pain which adversely affects the health and welfare of affected women [2,4,5]. Endometriosis is a considerable burden on the quality of life (QoL) of patients and their relatives, with increased medical cost and loss of productivity [6,7].

The molecular etiology of endometriosis is still largely unclear, with numerous factors been proposed to be implicated in its pathogenesis. Although endometriosis is linked with inflammation and immunological dysfunctions, it has not been shown to be an autoimmune disease [8].

Endometriosis presents itself in various forms and numerous classifications have been suggested for the lesions [9]. The most commonly used disease classification in endometriosis is provided by the American Society of Reproductive Medicine (ASRM) [10,11] that provides a guideline for recording the pathological findings and assigning the disease status [12,13]. Points are distributed based on the propagation of the endometrial-like tissue, its penetration depth in the ectopic sites, and the affected areas of the body.

Existing practice largely depends on laparoscopy for a definitive diagnosis, which often results in prolonged delays between the onset of the symptoms, diagnosis, and subsequent treatment [7].

There is no definite cure for endometriosis and the existing therapeutic strategies which includes surgery and pharmacological therapies that aim to relieve symptoms or enhance fertility are not sanative and often do not relieve symptoms [14,15].

The quantity of data being collected digitally and stored in the medical field is extensively vast and rapidly expanding. We currently have the capability to rapidly generate, store and analyze data that, just a few years ago, would have taken many

years to compile. As a result of these massive quantities of data accumulating from patients and the populations at large the term *big data* was coined in the 1990s to describe this phenomenon. However, big data no longer means what it once did. In 2016 the term was expanded to mean not just large data volume, velocity and variety but also our increasing ability to analyze and interpret them [16]. In addition there have been recommendations that for *big data* to be effective nuances such as quality, veracity and value needs to be considered [17,18]. The age of *big data* started in 2002, generating increased amount of alphanumeric data and has been successfully implemented in other aspects of everyday life. It is essential to understand that data on by itself is useless unless analyzed, interpreted, and acted on [19]. Big data in healthcare now contain quantitative data (*e.g.*, laboratory values), and qualitative data (*e.g.*, text-based documents). Nevertheless, a considerable amount of this dataset is perceived as a result of health care provision, instead of a crucial resource to improve its efficiency [20].

For so long *big data* has been stated to be a transformative force in modern medicine but it is important to remember that data should be appropriately analyzed, interpreted, and acted on to be of any value. Therefore algorithms and not *big data* will be the transformative force in modern medicine.[19] An algorithm is a process or a list of rules to be followed to solve a problem. Machine learning (ML) enables machines using algorithms learn concepts from experiences such as knowledge, data etc. Most computer-based algorithms in medicine are encoded knowledge on a particular subject, that are applied to draw conclusions on specific medical conditions and apply them to new patients [19].

Our long-term aim with this study was to discover innovative new tools for efficient diagnosis of endometriosis through the development of non-invasive diagnostic methods using the extensive amount of data in our Endomet database generated over the years. As well as to create tools to analyze and interpret this data that in turn could help understand the etiology and pathogenesis of the disease.

2 Review of the Literature

2.1 Endometriosis

Endometriosis is a benign, progressive, and sometimes aggressive gynecological disorder with individual variability and contrasting pathophysiologic processes. A broad range of clinical challenges that succeeds endometriosis for years has infuriated gynecologists, intrigued pathologists, and encumbered patients [21]. Although endometriosis was first described in 1860, Sampson's study in the 1920s had been the first to highlight the association between the clinical manifestations and pathological findings of endometriosis [22]. By definition endometriosis is the presence of endometrial glands and stroma outside the uterine cavity in ectopic locations [1], it is often described to occur mainly in three areas in the pelvis, on the ovaries; in the peritoneum; and in the rectovaginal septum. These different subtypes are often referred to as ovarian endometrioma or cysts (OMA), superficial peritoneal implants or lesions (PeL), and deep infiltrating endometriosis lesion (DiE) **Figure 1**. Endometriosis rarely spreads to extra-abdominal organs but has been documented to manifest in numerous other localizations outside the pelvic region (except the spleen) [23], including the brain [24], lungs, diaphragm, lymph nodes, knee [25], the gastrointestinal system, the thorax, urinary tract and the nasal mucous membrane [26], as well as rare cases of vesical endometriosis, that occurs in 1% of women, particularly during pregnancy [27]. Also there have been instances of hepatic endometriosis cited in literature [28,29].

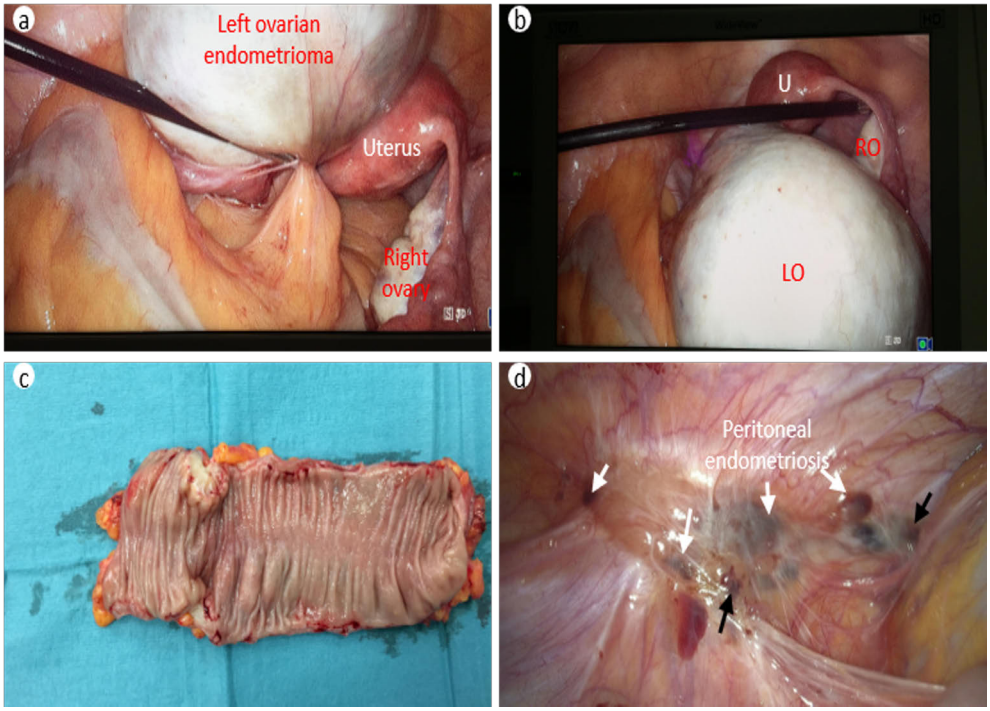


Figure 1. Laparoscopic appearance of the most common types of endometrioses. (a, b) left ovarian endometriotic cysts, (c) deep infiltrating lesion in the bowel (d) peritoneal lesion. Lesions are shown in arrows, U = uterus, LO = ovary with endometriotic cyst, RO = right ovary. Pictures taken by Pia Suvitie M.D., Ph.D.

Additionally, endometriotic lesions have been reported to be found on the uterine ligaments, the cervix, vulva, abdominal wall, vagina, and labia [30,31] **Figure 2**. It is a widespread, inflammatory disorder that is manifested primarily in women from pro menarche until post menopause, irrespective of race, ethnicity, or maternal status. As such, endometriosis as a disease can be categorized as either endopelvic or extrapelvic [26]. Endometriosis has multifactorial pathogenesis which includes genetic, hormonal, and environmental factors. In a large proportion of women between the ages of 20 and 50, the exposure to ovarian hormones play a crucial role as estrogen stimulates the growth of endometriosis and anomalies in estrogen signaling have been linked with the disease [32,33]. Although, it affects all races and ethnicities, when compared to women of Asian or African genetic origin, Caucasians are more prone to suffer from endometriosis [34], with an increase in prevalence among tall women and those with low Body mass index (BMI) [35]. About 51% of the associated risk of endometriosis can be linked to genetic factors as there is a 7 to 10-fold probability of developing endometriosis with a family history of endometriosis or an affected relative [36,37].

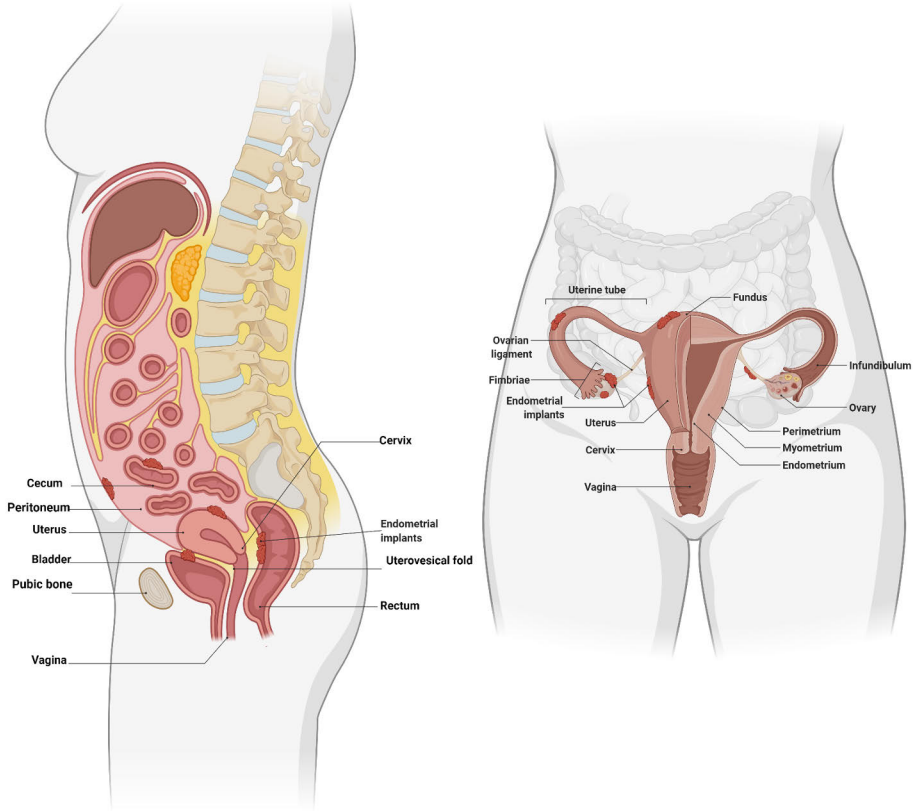


Figure 2. Sagittal section and anterior view of the uterus showing typical location of endometriosis lesions. Created in BioRender.com.

The prevalent characteristics of endometriosis in patients is truly indicative of the metastatic trait of cancer, which endometriosis is frequently compared to [38]. The prevalence of endometriosis is difficult to identify, in part due to the variability in its clinical manifestation. The ability to discover endometriosis not only in the abdominopelvic cavity, but also in the thoracic cavity as well as the in the nasal mucosa are indications that there are several processes involved in the pathogenesis of endometriosis. Also a reliable and definite diagnosis can only be made at surgery via laparoscopy, when the endometriotic lesion can be confirmed visually and histologically and that comes with well recognized risks [39,40]. The heterogeneity of endometriosis also increases the possibility of false negative laparoscopic surgery in symptomatic women, while the absence of implantations in the pelvic cavity might not necessarily mean the lack of endometriosis foci in other regions of the body [38]. The prevalence of endometriosis is reported to be around 1.5% in some population based studies compared with clinical based studies that report it to be between 8 and

15% [41], while other large clinical / surgical cohort studies estimate it to be around 2 and 10% [42] of reproductive aged women. Thus, the prevalence could be as high as an estimated, 176 million women worldwide [43,44]. Endometriosis can be asymptomatic but when symptomatic the effects of endometriosis can have prolonged adverse effects on the mental health, individual relationships, quality of life and work productivity of affected patients [2,4,5,45].

Diagnostic delays in endometriosis among affected women are well-documented and they vary from country to country, the delay ranges from between 7 to 12 years [46–50] with an estimated 6 of 10 endometriosis cases left undiagnosed [51]. As a result, these women may be experiencing the effects of endometriosis without having the benefit of understanding the underlying cause of their symptoms [52]. The economic burden of endometriosis as a disease is as high or like other chronic diseases such as Crohn's disease, diabetes, and rheumatoid arthritis. The average annual societal cost associated with endometriosis is estimated to be around €10,000, in an European study of 10 countries with at least two-thirds of the costs from the loss of productivity [5,6]. In the United States the annual societal cost is estimated to be from between $18.8 to $22 billion [53,54]. There are no serum markers available today that can effectively diagnose endometriosis. CA-125 which is widely used can have elevated levels in endometriosis, yet, it has quite limited clinical efficacy as concentrations of CA-125 can also be elevated in other conditions.

Endometriosis is commonly associated with infertility, as several studies have demonstrated that women with endometriosis often have decreased ovarian reserve predicated on the low levels of anti-Mullerian hormone [55]. Multiple mechanisms other than the direct effect on the ovary and its reserve could also influence infertility in endometriosis patients including adhesions, ovarian cysts, and changes in tubal anatomy [56–58]. The excessive production of inflammatory mediators can also lead to unsatisfactory function and damage to the oocyte [59]. As there is no known cure, treatment, or management aim to alleviate symptoms or enhance fertility.

2.1.1 Clinical presentation, symptoms, and treatment

2.1.1.1 Clinical presentation

Endometriosis in its clinical presentation is characterized by 1) its appearance, 2) by the location of the lesion in the pelvic region or abdomen and 3) by the extent of the disease. The symptoms and their severity vary greatly as well as the impact on the physical, social, and mental health of affected women. Most women with endometriosis will present a compendium of symptoms. Although, the gravity of symptoms and in particular pain do not correlate directly with the extent of the disease. Analogous to the normal endometrium the endometrial lesion possess the

same gonadal sex steroid receptors and the growth of the lesion is heavily dependent on estrogen stimulus [60]. The lesions therefore react to normal cyclic hormonal environment. The microscopic internal bleeding, together with the resulting inflammatory response, neovascularization, and the formation of fibrosis, are responsible for the clinical symptoms [21] experienced by patients.

The ovarian endometrioma (OMA) appears as a dark fluid filled cyst (containing blood and endometrial tissues) also known as chocolate cyst. The superficial peritoneal endometriosis implants or lesions (PeL) are categorized by their appearance or morphology and can take on many visual appearances, mostly representing their vascularization and fibrosis. Peritoneal endometriosis implants were initially taught to be like powder burns or mulberry lesions of the peritoneum [61]. Recently numerous stages of the implant development have been discovered, with a corresponding appearance for each stage. Early, functional lesions appear like papule lumps or vesicles, with a range in color from clear to pink, or bright red [61,62]. Advanced, active lesions assume a more typical appearance identifiable at surgery. These lesions can convey a wide range of colors, from red, green, black, brown, to purple [61]. This is owing to the existence of heme degradation products in the lesion as it undergoes hemorrhage and fibrosis. Inactive and or healed lesions are either white or calcified in appearance representing the remnants of the glands embedded within the fibrous tissue [61,62]. Cyclic inflammatory reaction in the peritoneal endometriotic lesions can result in the peritoneum surface being creased or containing windows, a defect also known as Allen-Masters window, commonly found in women with endometriosis [61,63]. In the deep infiltrating lesions (DiE), the endometrial tissue is at least 5 mm under the peritoneal lining or has invaded organs outside the pelvic cavity. The deep infiltrating implants are often found in specific locations, which includes the bladder, rectum, bowels, ileum, appendix, and posterior area [64,65]. Posterior DiE can include the sacrouterine ligaments [66], the retrocervical area of the uterus where the uterosacral ligaments merge together (torus uterinus) [67], the anterior rectal wall and the posterior vaginal wall [65,68].

2.1.1.2 Symptoms

Establishing a definite diagnosis of endometriosis based on symptoms alone can be difficult because the clinical presentation varies as there are considerable similarities with other gynecological conditions such as pelvic inflammatory disease, adenomyosis, leiomyoma (fibroids), uterine myoma etc. and non-gynecological conditions such as interstitial cystitis (inflammation of the bowel interstitial), irritable bowel syndrome, inflammatory bowel disease etc. all of which can contribute to the symptomatology. Up to a third of endometriosis patients remain asymptomatic [69,70] with a vast majority being diagnosed during investigations for

infertility or other laparoscopic surgeries, such as tubal ligation [27,71]. Endometriosis symptoms can vary but often reflect the location and the depth of the endometrial implants. Pelvic pain, often described as chronic, cyclic, and persistent is one of the most common symptoms associated with endometriosis [72–75]. The pain experienced may be a result of multiple mechanisms but is not always cyclical and not always confined to the pelvis, sometimes it progresses and radiates to the lower back. A study by Laux-Biehlman *et al*. demonstrated how pelvic pain symptoms are caused by the menstrual fragments or debris in the peritoneal cavity through the activation of mast cells and macrophages which in turn then stimulates sensory nerve endings [76]. As 20% of endometriosis cases are asymptomatic, the biochemical pathway of nerve stimulation through the release of damage and pathogen-associated molecular patterns from the menstrual fragment or debris [76] do not apply, indicating that the inflammation caused by menstruating ectopic lesions and menstrual fragments or debris may not be the only cause of pelvic pain [38]. Typical symptoms also associated with endometriosis include dysmenorrhea (severe pain before and or during menstruation), deep dyspareunia (pain during sexual intercourse), dysuria (pain during urination), dyschezia (pain during defecation). Other conditions commonly associated with endometriosis include chronic fatigue, and infertility. The prognostic significance of any one symptom or a set of these symptoms remains ambiguous as each or one of these symptoms can have other causes.

Endometriosis is associated with impaired fertility, and an estimated 25-50% of women with infertility are diagnosed with endometriosis [77–79], with around 30-50% of women diagnosed with endometriosis having some degree of infertility [80,81]. The mechanisms connecting both endometriosis and infertility are still poorly understood with no established foundation. By disrupting the function of the fallopian tube, as well as embryo transport, and the eutopic endometrium, endometriosis can further impair fertility. Even in mild form endometriosis can still impair fertility as chronic inflammation with increased levels of several cytokines in the peritoneal cavity can constitute a hostile environment for sperm. Just as with the mild form the more severe form of the disease may result in tubal adhesions, reduced ovarian reserve, as well as the quality of embryo and oocyte [81].

2.1.1.3 Treatment

There is no known cure for endometriosis, and it can often be difficult to manage depending on the presenting symptoms or complaints. There has been a lot of research done in recent years, to develop new treatments for the management of endometriosis. All existing guidelines [82] currently in use, are largely based on whether or not the patient is attempting to get pregnant in the near future and alleviating symptomology so conditions don’t interfere with everyday life.

Numerous factors such as age, severity of symptoms like pain, severity of disease, reproductive plans, medical history, side effect profiles, cost and accessibility are often considered when determining both medical and surgical management for endometriosis symptoms. Current management guidelines recommend experiential medical therapy before a definitive surgical diagnosis if present with typical symptoms [3,83–85]. There are several medical treatments available for the management of endometriosis symptoms and they are all aimed at reducing symptoms, fertility preservation, and preventing recurrence, thereby prolonging the time between surgery, or eliminating the need for surgery or repeat surgeries. Unfortunately, there are extremely limited treatment options when there is a desire for both pregnancy and treatment of pain symptoms. Endometriosis requires sustained management and for decades, the strengths of conventional endometriosis management have been non-steroidal anti-inflammatory drugs (NSAIDs) and combined oral contraceptives pills (COCPs) followed closely by gonadotropin-releasing hormone (GnRH) agonists, oral progestins, aromatase inhibitors and danazol [83,84]. Not all treatments work well, and symptoms may return if medical therapy is stopped or if more time elapses after surgery in the case of surgical management.

2.1.1.3.1 Medical therapy

NSAIDs are the most commonly prescribed analgesics for endometriosis associated pain symptoms, and despite their widespread use, when compared with placebos there is very little evidence for their efficacy [86]. NSAIDs act by blocking the cyclooxygenase (COX) enzyme which is vital in the production of inflammatory mediators. COX accelerates the conversion of arachidonic acid to PGH2 that is converted into PGE2 and PGF2α via the action of PG synthetase [87]. Although studies have shown the concentration of COX-2 enzyme to be relatively higher in the ectopic endometrial tissues [88–91] COX-1 and COX-2 enzymes are both present. As well as unusually high concentrations of PGE2, PGF2α, and other prostaglandins in the uterine tissues of women who suffer from extremely heavy menstrual bleeding (menorrhagia), dysmenorrhea, or endometriosis [92–94].

There is no obvious evidence of one medical treatment option having advantage over another, although hormonal medication therapies constitute the first option in many cases for patients plagued by endometriosis. The goal with hormonal treatments is to suppress ovulation and stimulate local hypoestrogenic state. This reduces the conversion of arachidonic acid to prostaglandins, as a result slowing the growth or progression and the activity of both the endometrium and the endometrial lesions therefore stabilizing steroid hormone milieu and lessening disease activity and pain. Continuous administration of hormonal medication seems to be a more effective way of reducing the recurrence of dysmenorrhea but not the noncyclic

pelvic pain or intercourse pain [95]. The associated cost, the convenience of administration and tolerability are some of the principal elements that have contributed to the popularity for the use of hormonal therapy. A systematic analysis on the effectiveness of COCPs and continuous progestogens, which includes dienogest, medroxyprogesterone acetate, cyproterone acetate, or norethisterone, corroborated the efficacy of these approach for endometriosis associated pain symptoms [86,96] **Table 1**. No means of administration (oral, transdermal, or transvaginal) has been demonstrated to deliver improved pain alleviation and some limiting factors for the use of hormonal medication include long-term administration, reduced fertility due to contraceptive effect, high risk of thromboembolism, and high rates of recurrence after discontinuation.

Table 1. Treatment options for endometriosis associated pain modified from Carpinello OJ *et al*. 2000. [61]

Treatment agent	Administration route	Side effects
Combined hormonal contraceptive	Oral	Mild nausea, vomiting
Progestins	Oral, Injection, or Intrauterine	Breakthrough bleeding, Breast tenderness, Bone mineral density and lipid profile in some, Androgenic side effects in others.
GnRH agonists	Injection, or Intranasal	Symptoms of hypoestrogenic state (Hot flashes, vaginal dryness, mood irritability, sleep disturbances, and decreased bone mineral density)
GNRH antagonists *	Oral	Symptoms of hypoestrogenic state + unfavorable changes in the lipid profile
Aromatase inhibitors	Oral	Ovarian stimulation in pre-menopausal women, hypoestrogenic effects
Danazol **	Oral	Weight gain, breast atrophy, fluid retention, hirsutism, hot flushes, acne, oily skin
Gestrinone	Oral	Weight gain, hirsutism, seborrhea, unfavorable changes in the lipid profile, and acne
Prostaglandin Inhibitors	Oral	Unfavorable gastrointestinal side-effects

* GnRH antagonists are not yet approved by the Food and Drug Administration (FDA) or the European Medicines Agency (EMA) but may be a potential future treatment choice. ** Danazol no longer in Finnish market – available only by special permit.

Progestins only therapy is often preferred in women with a greater risk for stroke, myocardial infarction, or thrombotic incidents and women for whom combined hormonal therapy has not worked. Progesterone stimulates decidualization of the endometrium, alters estrogen receptors, reduces estrogen induced mitosis, reduces angiogenesis and the expression of matrix metalloproteinase needed for lesion growth [97,98]. All these processes form the pathophysiologic core for the use of progesterone in the treatment of endometriosis (for review, see [99–101]). Several of the progestins used include dienogest, cyproterone acetate, dydrogesterone, lynesterole, gestrinone, megesterol acetate, medroxyprogesterone acetate, norethindrone acetate and drospirenone. Most are administered orally, while others are administered through other routes; intramuscularly/subcutaneously or by intrauterine route. Of all dienogest (a 19-nortestosterone derivative), has high-level specificity for progesterone receptors and fewer antiandrogenic side effect profile and when continuously administered this leads to decidualization and atrophy of the endometrial lesions. Additionally it has anti-inflammatory, as well as anti-angiogenic and anti-proliferative effects [83,102–105]. Dienogest has also been shown in several studies for safety and efficacy to have a positive profile, it is well tolerated in general and adverse reactions involve bleeding irregularities, which improves with time. Patients have reported improvements in symptoms associated with endometriosis and a general improvement in quality of life [106,107].

Although, progestins has a much improved side effect profiles than combined hormonal therapy [108], their use is associated with limitations such as the continuous need for compliance with usage, unscheduled bleeding and spotting even with correct usage etc. Multiple studies have demonstrated the effectiveness of Levonorgestrel releasing intra-uterine system (LNG-IUS) a T shaped intrauterine device (IUD), which delivers 20 micrograms of progestin daily locally over a five-year period without systematic side effects. Vercellini *et al*. showed LNG-IUS to effectively regulate endometriosis associated pelvic pain and improve patient satisfaction [109]. In another study that compares LNG-IUS to GnRH antagonists administration, comparable effectiveness was reported for both treatments, with lower prevalence of hypoestrogenic side effects in women using the IUD [110]. LNG-IUS has also been demonstrated in other studies to help reduce the recurrence rate of dysmenorrhea among women following laparoscopic surgery for symptomatic dysmenorrhea. Because of its better side effect profile with long term use, LNG-IUS offers a great option for women who do not desire pregnancy [111].

Among hormonal therapy, GnRH agonists have been suggested to be an effective treatment option against endometriosis associated pain symptoms [112]. GnRH agonists work by down regulating gonadotrophin release, inhibiting ovarian estrogen production resulting in endometriotic implants regression. After the initial phase of administration there is stimulation of the pituitary that releases follicle

stimulating hormone (FSH) and luteinizing hormone (LH), however, prolonged use results in the down regulation of pituitary GnRH receptors and a suppression of hypothalamic pituitary ovarian axis which is why they are approved for up to six months of continuous use only. The resulting estrogen deficiency induces adverse bone effects such as decrease in bone mineral density (BMD), vaginal dryness and atrophy, abnormalities in lipid profile and hot flashes [113]. GnRH agonists provide a high rate of pain relief [114] and are a great choice for those who initial treatment did not work for or are not suitable candidates for combined hormonal contraceptives and continuous progestogens due to their medical history. If GnRH agonist therapy is successful, the addition of add- back therapy such as hormone replacement therapy (HRT) can help decrease the rate of BMD and provide symptomatic relief. GnRH antagonists on the other hand do not result in initial flare ups as well as have a lower degree of hypoestrogenism and improved side effect profile when compared with GnRH agonists with equivalent symptomatic improvement as initial reports suggest dose dependent decrease in estrogen levels.

Alternative therapeutic agents for endometriosis management include danazol, aromatase inhibitors, selective progesterone modulators (SPRMs), and selective estrogen modulators (SERMs). Danazol is an androgenic agent that inhibits the surge of LH and ovarian enzymes to decrease the steroidogenesis of the ovary. However, it is seldomly used despite its effectiveness in controlling endometriosis related symptoms because of unfavorable androgenic side effects. These effects include hirsutism, acne, weight gain, liver dysfunction, muscle cramps, deepening of voice, and an anomalous lipid profile hence it has been taken off the market in several countries.

The aromatase enzyme converts steroid precursors into estrogen. Though the ovaries and fat are the primary source of the enzyme it is not present in the endometrium, but overly expressed in endometriosis [115]. Unlike GnRH agonists – blocking gonadotropin dependent estrogen synthesis, aromatase inhibitors prevent estrogen synthesis in both the ovaries as well as in the periphery. This is especially useful for postmenopausal women with endometriosis where the peripheral fat is the primary source of estrogen. Aromatase inhibitors also decreases aromatase activity within the endometriotic tissue and thus suppresses COX-2 activity, and decreases PGE2 levels [116–119]. When used in combination with combined oral contraceptives, progesterone or GnRHs, aromatase inhibitors have been demonstrated to substantially reduce the size of the lesions and endometriosis associated pain symptoms as well as improve QoL. However, with long term use its side effect profile include ovarian follicular cysts [120] which in practice is not an issue, rather the consequence of hypoestrogenism in treatments that do not include estrogen added with i.e. combined oral contraceptives. Both the SPRMs and SERMs are an emerging

class of therapeutic agents and they both have the ability to target the endocrine action of endometriosis (for review, see [121–137]).

New therapeutic options for endometriosis are needed given the limitations in the currently available management options. Endometriosis is predominantly diagnosed in reproductive aged women and all available treatment options interfere with fertility. The long-term use of hormonal therapy, prolonged hypoestrogenism and the high rates of recurrence following discontinuation are some of the limitations that needs to be addressed with new therapy options. Future therapeutic options may include medical therapies like statins; anti-angiogenesis factors; TNF-α blocker; peroxisome proliferator activated- receptor gamma ligand (PPAR-γ); and pentoxifylline (for review, see [120]) and non-invasive therapies such as high-intensity focused ultrasound (HIFU).

2.1.1.3.2 Surgical management

The surgical management of endometriosis is an effective alternative to medical therapy, after the failure of empiric therapy, or intolerance to medical therapy or for diagnosis and immediate treatment (**Figure 3**).

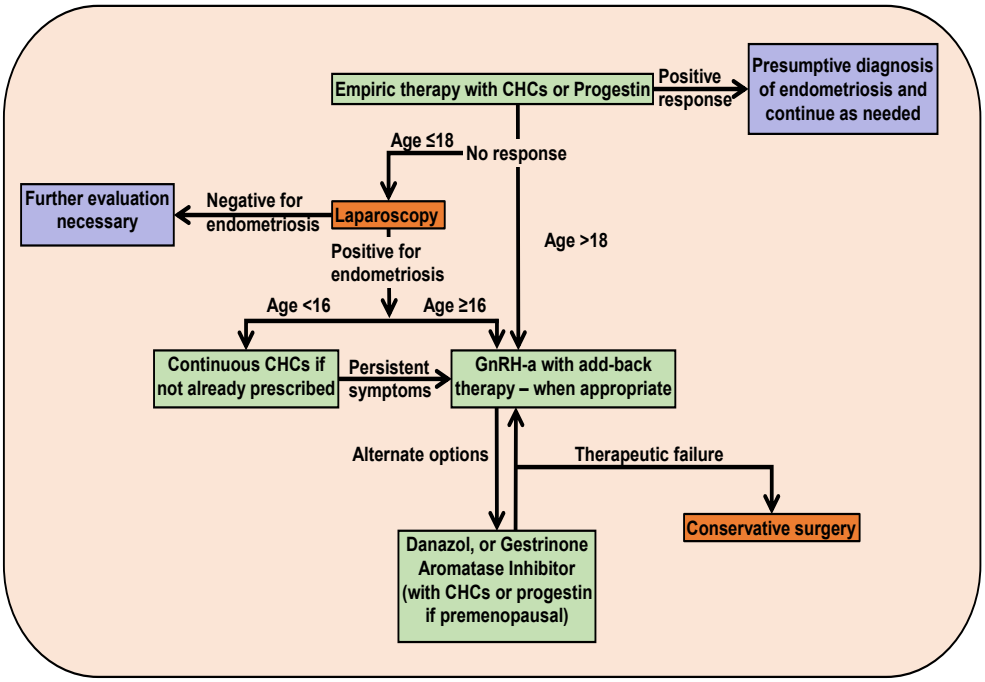


Figure 3. Treatment algorithm for suspected endometriosis and chronic pelvic pain. Combined hormonal contraceptive (CHC); Gonadotropin-releasing hormone agonist (GnRH-a). modified from Pharmacotherapy A Pathophysiologic Approach, 9th Ed. [138]

Choosing between medical therapy or surgical management depends on the extent of the disease and the presence of endometriosis lesions in adjoining organs like the bladder, bowel, appendix or the ureter [139]. Laparoscopy is the preferred surgical method for endometriosis surgery as it offers better visualization, reduces post-operative discomfort, with quicker patient recovery, and a return to normal activity as it decreases overall pain at 6 and 12 months compared to laparotomy [140,141]. As endometriosis is primarily diagnosed in reproductive aged women, those with endometriosis related pain symptoms who wish to preserve their fertility or desire spontaneous pregnancy surgical management is encouraged considering all medical therapies currently in use interferes with spontaneous ovulation. The main goal in endometriosis surgical management is to restore normal anatomy and to relieve pain. However, as with medical therapy, pain recurrence is common 21.5% of women within 2 years and in 40-50% within 5 years [142]. In many of these cases further surgery is needed [142–144]. Surgical management can either be conservative with endometriosis treatment or definitive with hysterectomy along with or without the removal of the ovaries for women not longing for pregnancy. Although, surgical management may temporarily help relieve pain, unfortunately it can result in various complications depending on the lesion type and where it is removed from [145–147]. A systematic review of randomized trials described the efficacy of surgical therapy for pain associated with superficial endometriosis [148,149], which can either be excised or ablated, with no clear statistical difference in pain scores, although there was a trend that favored excision of the lesion [150]. Some clinical studies confirm that, predominantly in women with bilateral disease surgery for endometriomas tend to reduce their ovarian reserve, this can have some adverse effect on fertility as ovarian stimulation might be compromised due to the reduction in ovarian reserve for women undergoing in-vitro fertilization (IVF) treatment [151]. In some observational studies there were indications that hysterectomy with bilateral salpingo-oophorectomy was a successful approach for managing endometriosis related symptoms in women who were not pursuing spontaneous pregnancy as surgical menopause is induced. Data from some retrospective study suggest that hysterectomy with ovarian preservation decreases endometriosis related pain symptoms, and only about a third will need additional surgery for symptoms after 5 years. By comparison 10% of those who undergo hysterectomy with oophorectomy for endometriosis will require further surgery. Endometriosis surgery should be centralized as it is very demanding and repeat procedures should be avoided. The risk of recurrence is high and hormonal treatment should be continued to minimize recurrence risk.

2.1.2 Pathogenesis of endometriosis and disease classification

2.1.2.1 Pathogenesis

Endometriosis is one of the most enigmatic gynecological diseases. The pathogenesis and etiology of endometriosis remain largely unknown and are still poorly understood, all endometriosis phenotypes can manifest within the same patient. There is no unifying theory regarding the origin of endometriosis but rather a few proposed theories (Sampson's theory; Meyer's theory; and Halban's theory) (**Figure 4**) that try to explain the pathophysiology of the disease, and none has been entirely proven or fully explain all endometriosis phenotypes [69].

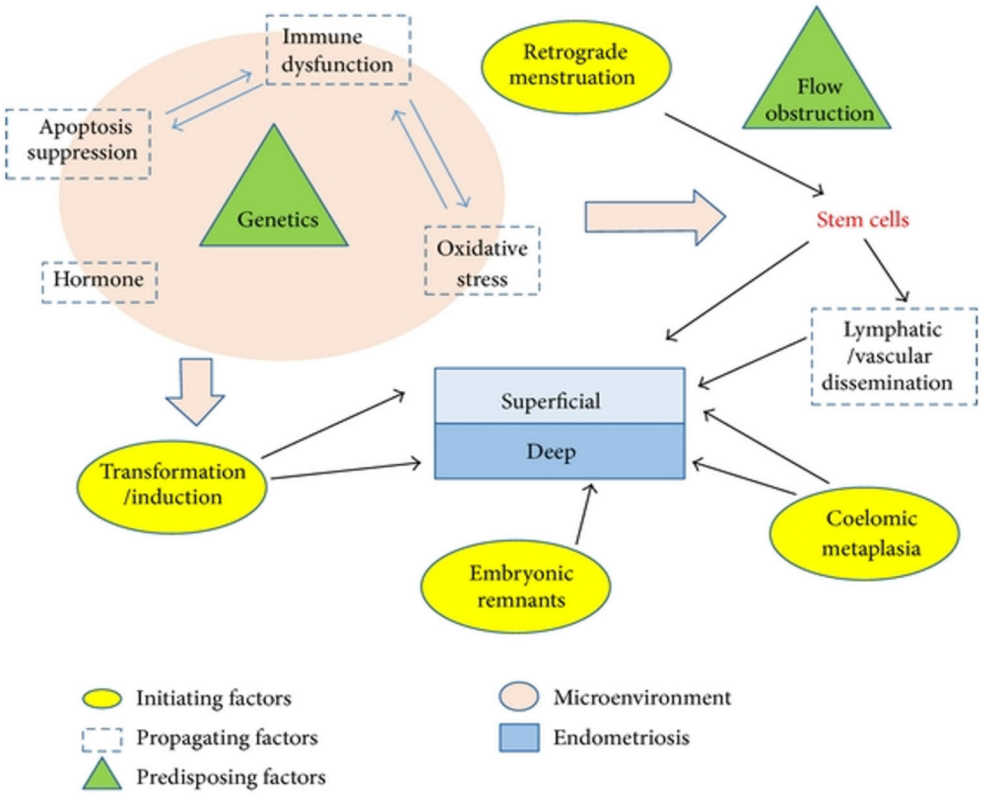


Figure 4. Proposed theories on the pathogenesis of endometriosis. The interaction between the various factors that may be involved in the pathogenesis of endometriosis. The different shapes indicate the initiating, propagating, and predisposing factors, respectively. Reprinted with permission from Samer Sourial *et al* 2014 [152] under the Creative Commons Attribution License.

The proposed theories include retrograde menstruation, altered immunity, vascular and lymphatic dissemination of endometrial cells, metaplasia of the germinal epithelium, embryonic rest theory, and the theory of metastatic spread (coelomic metaplasia theory) which could account for the pathogenesis of ovarian endometrioma and rectovaginal endometriosis [14,153]. Some recent studies have also suggested genetic origins and stem cell as factors that play a role in the development of the disease due to the high incidence in the families of affected women [81,154]. Even though a plethora of genetic factors have been predicted to be associated with an increased predisposition to endometriosis [155–157], a widely accepted gene for endometriosis is still far from being identified. Additionally, some other studies have suggested a variety of exposure factors to be linked with endometriosis pathogenesis. The theory being, that these exposure factors contributes to the development of endometriosis by disrupting the immune-mediated mechanisms, that stimulates the production of pro-inflammatory cytokines [158]. (**Table 2**)

Table 2. Role of the various theories in endometriosis pathogenesis modified from Samer Sourial *et al* 2014. [152]

Theory	Mechanism
Retrograde menstruation	Flow of endometrial content into the pelvis, allowing for implantation of endometrial lesions
Metaplasia	Transformation of peritoneal tissue/cells into endometrial tissue through hormonal and/or immunological factors
Hormones	Estrogen-driven proliferation of endometrial lesions. Resistance to progesterone-mediated control of endometrial proliferation
Oxidative stress and inflammation	Recruitment of immune cells and their production of cytokines that promote endometrial growth
Immune dysfunction	Prevention of eliminating menstrual debris and promotion of implantation and growth of endometrial lesions
Apoptosis suppression	Promoting survival of endometrial cells and downregulation of apoptotic pathways
Genetic	Alteration of cellular function that increases attachment of endometrial cells and evasion of these cells from immune clearance
Stem cells	Initiation of endometriotic deposits by undifferentiated cells with natural ability to regenerate

The Sampson's theory of retrograde menstruation is the earliest and the most accepted theory and the one to date supported by nearly all evidence explaining the etiology of endometriosis. This theory was derived in the 1920's from observations

made during surgeries. it suggests that endometriosis is caused due to the retrograde flow of shed endometrial tissue through the fallopian tubes into the peritoneal cavity during the menstrual period [159–161]. Perhaps by differential pressure originating from dyssynergic uterine spasms [15]. When in the peritoneal cavity, the regurgitated endometrium can embed itself in the pelvic structure and continue to grow. Epidemiological factors that would enhance pelvic contamination can increase the probability of this event. Factors such as early menarche, long-lasting menstrual flows, and any molecular alteration that favors the process of cell implantation and growth at ectopic locations (**Figure 5**) [15]. Although, the retrograde menstruation is said to occur in around 76 - 90% of women with patent uterine tubes not every one of these women have endometriosis [152,161,162]. This theory was further tested in non-human primate models, where endometriosis was induced by inoculating autologous menstrual endometrial tissue into baboons and macaques to stimulate retrograde menstruation in the peritoneal cavity [163,164]. Additional evidence to substantiate the Sampson's theory is derived from an observation that factors, such as congenital defects including iatrogenic cervical stenosis and imperforate hymen, would increase the risk of retrograde menstruation and the development of endometriosis [152,165].

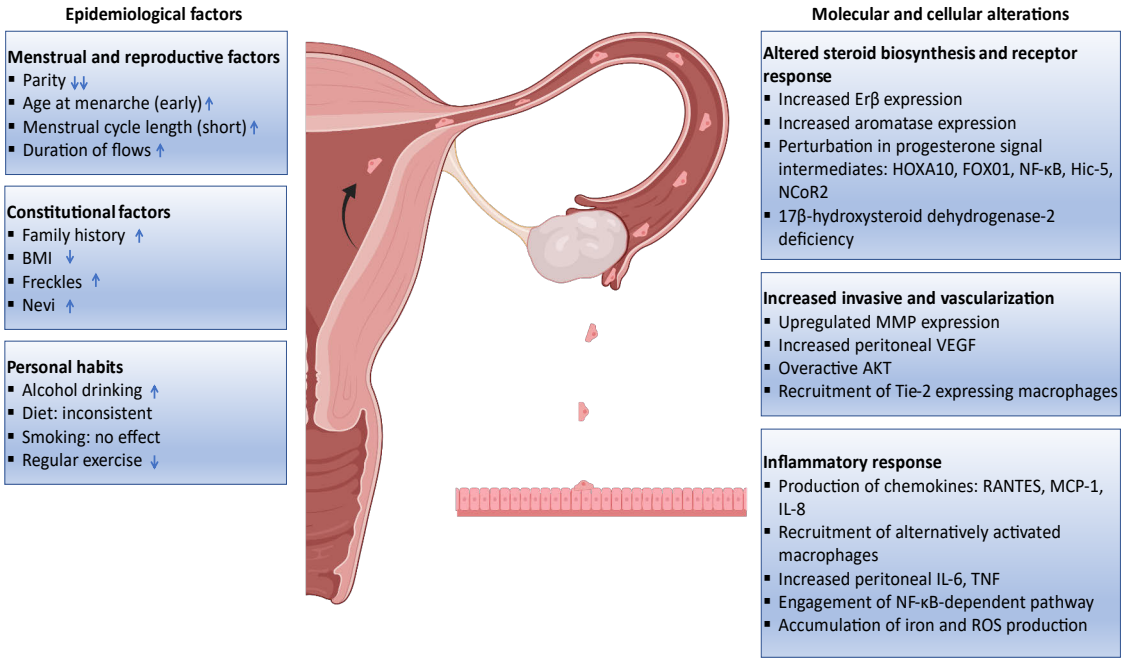


Figure 5. The Epidemiologic factors and the molecular mechanisms involved in endometriosis development modified from Vercellini, P. *et al.* [15]

The altered immunity theory is based on observations that among women with endometriosis, the prevalence of autoimmune disorders is increased. This supports the assumption that the pathogenic mechanism of endometriosis may involve defective immune responses in these women [166]. The regurgitation of endometrial cells into the peritoneal cavity activates an inflammatory reaction, that recruits locally activated macrophages and lymphocytes [167]. This inflammatory reaction can cause a defective immunosurveillance that inhibits the removal of the shed endometrial cells and stimulates the growth and implantation of the endometrial cells in ectopic locations [168]. Both the endometrial and the associated immune cells produce growth factors and cytokines, which induces angiogenesis and cellular proliferation; thus, modulating the implantation and growth of ectopic lesions [169]. Moreover, in the endometrium of endometriosis patients the function of natural killer (NK) cell is repressed in the endometrium of endometriosis patients, and evidently these cells are engaged in the identifying and obliterating foreign cells in the body [170,171].

In the vascular and lymphatic dissemination theory, the endometrial tissue moves through the vasculature or the lymphatic from the uterus and deposits in other places in the peritoneal cavity. Six to 7% of women who had undergone lymphadenectomy, were found to have histologically confirmed endometriosis. It has been proposed to account for the occurrence of endometriosis at atypical locations such as the lymph nodes, parenchyma of the lung, bone, and brain [165].

The coelomic metaplasia theory postulates, that the eutopic endometrial tissue is believed to originate from coelomic epithelia cells which undergo metaplasia or a metaplastic reaction [172]. Basically, the coelomic cells develop into peritoneum cells and surface of the ovaries, nevertheless here the coelomic cell undergo metaplasia and causes the cells to transform into endometrial cells, nonetheless the cells are not present in the uterus but rather they become present outside in the peritoneal cavity. The coelomic metaplasia could explain the incidence or occurrence of endometriosis in women with Mullerian agenesis, who have an absent uterus [173] or prepubertal girls as estrogen which is fundamental for endometrial growth is absent [174] as well as the occasional presence of endometriosis in men [175]. Ectopic endometrial tissues has also been found in female fetuses as a result of defective embryogenesis [152].

Von Recklinghausen and Russell [176,177] pioneered the embryonic rest theory in the 1890s. The theory states that the Müllerian embryonic cell rests could differentiate into functioning endometrium under certain stimulus. The Müllerian embryonic cells from the Müllerian ducts, migrate into the peritoneal cavity and develop into endometriotic lesions that responds to estrogen [152,165]. In the coelomic metaplasia theory, the provenance of endometriosis is essentially limited to the mesothelium, while the embryonic rest theory suggests that endometriotic lesions originate from embryonic cell rests that are not necessarily restricted to the

mesothelium. Müllerian embryonic cell rests are not just present in women but also in men, this could very well explain the very rare case of endometriosis reported in men [173,178–183]. Nisolle and Donnez hypothesized that the occurrence of rectovaginal endometriosis is linked to an adenomyotic nodule derived from the Mullerian rests through metaplasia [14,153].

The endometrium undergoes cyclic regeneration all through the reproductive age, which suggests that the endometrium holds stem/progenitor cells. Recent studies support evidence of the existence of endometrial stem/progenitor cells and their potential involvement in the regeneration and differentiation of eutopic endometrium [184–186]. By definition, stem cells are undifferentiated cells, typified by their capacity to regenerate and differentiate into a single or multiple types of specific cells. The role stem cells play in the creation of endometriotic debris may have been the result of irregular migration of normal endometrial basalis through retrograde menstruation [152,187]. A study by Leyendecker *et al*. postulated that the women who suffer with endometriosis unusually shed endometrial basalis tissue, which instigate the deposit of endometriotic debris after retrograde menstruation. In the case where the basalis contains stem/progenitor cells, they are more likely to survive and therefore initiate the deposit of endometriotic debris in the peritoneal cavity than differentiated endometrial cells from the functionalis [152,188].

Owing to their innate potential to rejuvenate, stem cells could give rise to the deposit of new endometriotic debris. Brosens *et al*. hypothesized that a large quantity of endometrial progenitor cells contained in the uterine bleeding in neonatal girls could endure in the peritoneal cavity long after retrograde flow and reactivate as a response to ovarian hormones in adolescents [152,189]. Nevertheless, the quantity of endometrial progenitor cells in neonatal bleeding has not yet been determined in comparison to the endometrium of adult women. Women who suffer with endometriosis probably shed considerably more stem-cell rich basalis layer when compared to women without endometriosis [152,188], which would support the likelihood of retrograde menstruation providing access for endometrial stem cells to flow through to extrauterine structures [152,188,190]. Another Possibility, is that these stem cells could be carried through the lymph nodes or vasculature to ectopic locations [191]. Another alternative possibility of the involvement of stem cell in endometriosis would be the lineage reprogramming of the hematopoietic, peritoneal, or ovarian stem cells into endometrium like tissue [152].

2.1.2.2 Disease classification

Although endometriosis has been recognized since the early part of the 20th century it remains an enigmatic disease. Several attempts at classification has been challenging and subject to much controversy due to its many manifestations and

heterogeneity, the focus on classification has remained largely on the anatomy, histology and disease burden for surgical staging and recently for prognostic value [9,192]. These ambiguities have resulted in classification schemes with inadequacies in symptom management, recurrence, and association with other disorders. An ideal classification system should accurately assess the disease state with reverence to the extent, the location, and the nature of the disease. Moreover, it should be useful in predicting the outcome in treatment response. The earliest endometriosis classification system was developed in 1921 by Sampson [193], and later other classifications systems followed, by Acosta *et al*. [194] and, in the German-speaking countries, by Albrecht *et al*. [195]. The American Fertility Society (AFS) score [196] was established later on in 1979, but became instituted as the most widely accepted classification system following its revision in 1985 (rAFS score) [197] (**Figure 6**). It was later renamed the revised American Society for Reproductive Medicine (rASRM) score in 1996 [10,11]. Since its revision, the rASRM still remains the most commonly used classification system for endometriosis despite its flaws [9,10]. There is a growing consensus that the current classification system needs to be overhauled. A good classification system should be a proper solution to improve the usefulness of disease classification in endometriosis related symptom management, association with other disorders, recurrence, projection for response to therapies, quality of life and other rudiments of significant importance to women with endometriosis or their healthcare providers [198]. The system should be simple and easy to execute; it should be scientifically and analytically based; inclusive for all cases; uses unambiguously distinct terminologies; allow for unassuming description of the disease; correlate well with the symptoms; give predictive information; envisage responses to treatment for pain, infertility and recurrence of symptoms after treatment [198,199]. In addition to the rASRM categorization, other promising classification methods include the Enzian-score classification for deep infiltrating endometriosis (DIE) [198,200,201] (**Figure 7**), and the endometriosis fertility index (EFI) [199,202] for predicting pregnancy after surgery (**Figure 8**).

AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE
REVISED CLASSIFICATION OF ENDOMETRIOSIS

Patient's Name _____ Date _____

Stage I (Minimal) - 1-5
Stage II (Mild) - 6-15
Stage III (Moderate) - 16-40
Stage IV (Severe) - >40
Total _____

Laparoscopy _____ Laparotomy _____ Photography _____
Recommended Treatment _____
_____
Prognosis _____

PERITONEUM	**ENDOMETRIOSIS**	<1cm	1-3cm	>3cm
	Superficial	1	2	4
	Deep	2	4	6
OVARY	R Superficial	1	2	4
	Deep	4	16	20
	L Superficial	1	2	4
	Deep	4	16	20
	POSTERIOR CULDESAC OBLITERATION	Partial	Complete	
		4	40	
OVARY	**ADHESIONS**	<1/3 Enclosure	1/3-2/3 Enclosure	>2/3 Enclosure
	R Filmy	1	2	4
	Dense	4	8	16
	L Filmy	1	2	4
	Dense	4	8	16
TUBE	R Filmy	1	2	4
	Dense	4*	8*	16
	L Filmy	1	2	4
	Dense	4*	8*	16

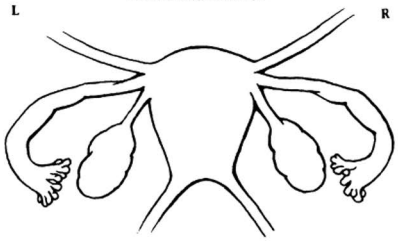
*If the fimbriated end of the fallopian tube is completely enclosed, change the point assignment to 16.

Denote appearance of superficial implant types as red [(R), red, red-pink, flamelike, vesicular blobs, clear vesicles], white [(W), opacifications, peritoneal defects, yellow-brown], or black [(B) black, hemosiderin deposits, blue]. Denote percent of total described as R___%, W___% and B___%. Total should equal 100%.

Additional Endometriosis: _____

Associated Pathology: _____

To Be Used with Normal Tubes and Ovaries



To Be Used with Abnormal Tubes and/or Ovaries

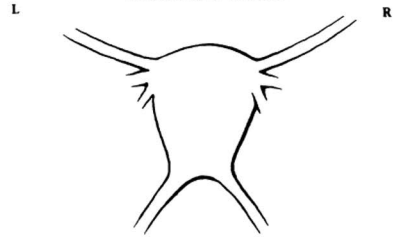


Figure 6. The revised American Society for Reproductive Medicine scoring system for all women with endometriosis. Reprinted with permission from Elsevier [11,198].

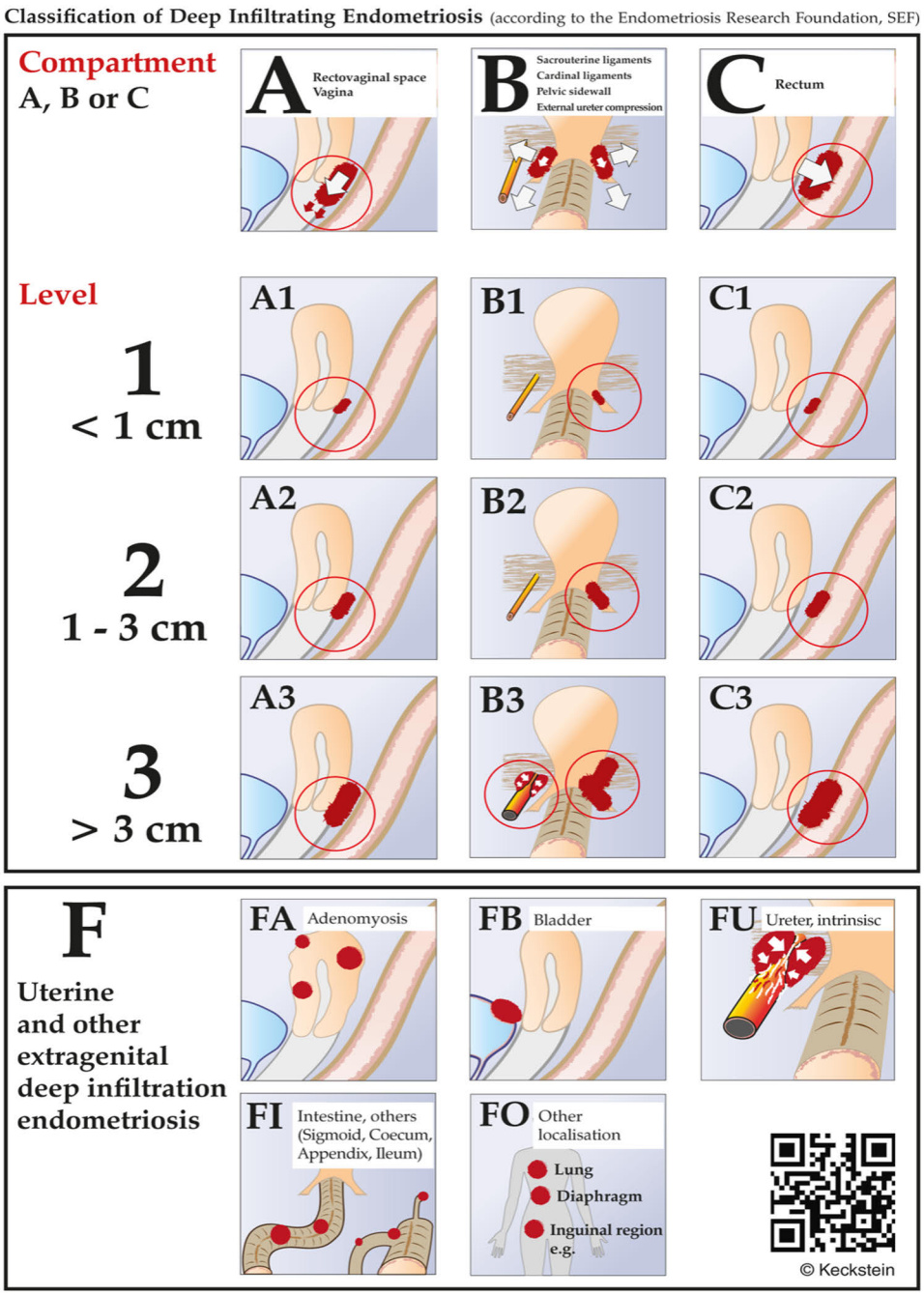


Figure 7. Enzian scoring system from Jörg Keckstein [203] for women with deep endometriosis. Reprinted with permission from Johnson *et al.*, 2017 [198].

ENDOMETRIOSIS FERTILITY INDEX (EFI) SURGERY FORM

LEAST FUNCTION (LF) SCORE AT CONCLUSION OF SURGERY

Score		Description
4	=	Normal
3	=	Mild Dysfunction
2	=	Moderate Dysfunction
1	=	Severe Dysfunction
0	=	Absent or Nonfunctional

To calculate the LF score, add together the lowest score for the left side and the lowest score for the right side. If an ovary is absent on one side, the LF score is obtained by doubling the lowest score on the side with the ovary.

	Left	Right
Fallopian Tube		
Fimbria		
Ovary		
Lowest Score	☐ Left +	☐ Right = ☐ LF Score

ENDOMETRIOSIS FERTILITY INDEX (EFI)

Historical Factors			Surgical Factors		
Factor	**Description**	**Points**	**Factor**	**Description**	**Points**
Age			LF Score ☐		
	If age is ≤ 35 years	2		If LF Score = 7 to 8 (high score)	3
	If age is 36 to 39 years	1		If LF Score = 4 to 6 (moderate score)	2
	If age is ≥ 40 years	0		If LF Score = 1 to 3 (low score)	0
Years Infertile			AFS Endometriosis Score		
	If years infertile is ≤ 3	2		If AFS Endometriosis Lesion Score is < 16	1
	If years infertile is > 3	0		If AFS Endometriosis Lesion Score is ≥ 16	0
Prior Pregnancy			AFS Total Score		
	If there is a history of a prior pregnancy	1		If AFS total score is < 71	1
	If there is no history of prior pregnancy	0		If AFS total score is ≥ 71	0
Total Historical Factors			**Total Surgical Factors**		

EFI = TOTAL HISTORICAL FACTORS + TOTAL SURGICAL FACTORS: ☐ Historical + ☐ Surgical = ☐ **EFI Score**

ESTIMATED PERCENT PREGNANT BY EFI SCORE

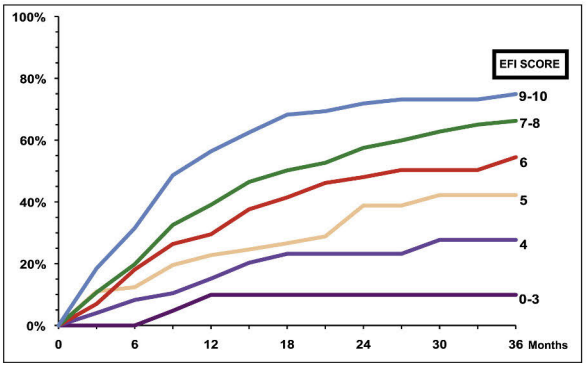


Figure 8. Endometriosis Fertility Index for women with endometriosis for whom future fertility is a consideration. Reprinted with permission from the American Society for Reproductive Medicine [199,202].

At the moment, all the techniques employed to categorize endometriosis deeply bear resemblance to a modular assembly system and all these categorization systems are too often criticized for their poor correlation with symptoms in addition to their dearth of predictive prognosis. Furthermore, the classifications do not provide a clear pathway for the treatment of pelvic pain and infertility. And at the same time, they look to classify appearance and size of the lesions, anatomical locations, the extent of pelvic adhesions and also predict fertility.

The rASRM classification system assigns values to endometriosis lesions in the peritoneum and ovaries using a point base system that corresponds to the size of the lesion(s). Points are also given for adhesions on the ovaries, the fallopian tubes and for partial or complete posterior cul-de-sac obliteration. All the assigned points are then summed up, and the final sum classified into four diagnostic categories representing the severity of the disease, stages I – IV (**Figure 9**). The stages are categorized as follow: Stage I (minimal); stage II (mild); stage III (moderate); and stage IV (severe) **Table 3**. However, the classification system does not effectively describe severity of pain, sterility and deep infiltrating endometriosis and it poorly correlates with QoL, the risk of recurrence or treatment outcomes [9,204,205].

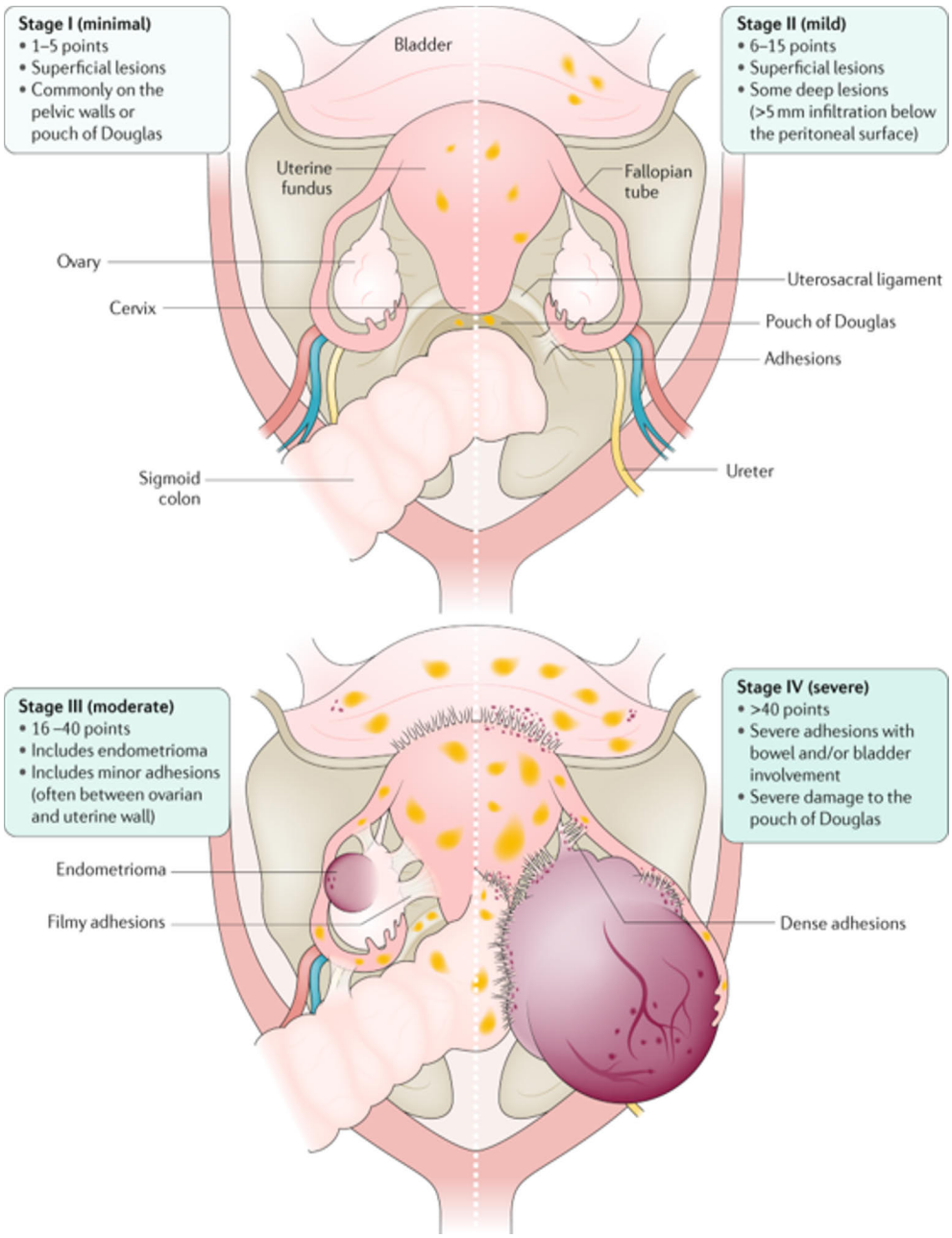


Figure 9. The revised American Fertility Society and American Society of Reproductive medicine endometriosis staging system based on a point system that considers location, extent and depth of disease in relation to pelvic structures[11]. Reprinted with permission from Zondervan *et al*., 2018 [206].

Table 3. Endometriosis stages.

Disease	Stage	Description
Minimal	**I**	Superficial lesions commonly on pelvic wall or pouch of Douglas
Mild	**II**	Superficial lesions and some deep infiltrating implants
Moderate	**III**	Deep infiltrating implants, endometriomas, on one or both ovaries, some minor adhesions (often between ovarian and uterine wall)
Severe	**IV**	Deep infiltrating implants, endometriomas on one or both ovaries, severe adhesions with bowel and/ or bladder involvement, severe damage to the pouch of Douglas

The Enzian classification has a comparatively excellent sensitivity and specificity for the diagnosis of surgical findings for DiE. It was introduced for the first time in 2005 to augment the rASRM score [200] and later revised in 2010 and 2011 [207]. The revised version combines morphological structures into 3 compartments: A. anterior (rectovaginal septum and vagina), B. lateral (sacrouterine ligaments and pelvic sidewall) and C. posterior (rectum and sigmoid colon) in order to simplify the system. The severity for all compartments is rated the same as follow: in Grade 1, the incursion is <1 cm Grade 2, the incursion is 1–3 cm Grade 3, the incursion is >3 cm. DiE outside the pelvis and the invasion of organs can be recorded separately. The Enzian classification also lists other distant locations such as adenomyosis (FA), involvement of the bladder (FB), intrinsic involvement of the ureter (FU), bowel disease cranial to the rectosigmoid junction (FI) and other locations, such as abdominal wall endometriosis (FO) [201]. As a consequence of the excellent morphological characterization provided, the estimation of laparoscopic operating time for DiE can also be calculated [208].

The rASRM categorization system unfortunately cannot effectively predict the clinical findings of treatment, particularly the pregnancy rate (PR) in infertile women. Adamson and Pasta [209] in 2010, suggested the use of the EFI as a new scoring system to predict spontaneous pregnancy after surgery when the patient has a functional gamete and uterus. The EFI considers the patient's age, length of infertility, and previous pregnancies. In addition, it uses the rASRM score and the least function score as the anatomical and functional result of the surgery on the fallopian tubes, fimbriae, and ovaries. The higher a patient's EFI score, the higher their chances of spontaneous pregnancy.

2.1.3 Endometriosis Diagnosis

Determining a definite diagnosis of endometriosis exclusively based on its clinical presentation is difficult due to the broad spectrum of symptoms, none of which are pathognomonic and frequently have similarities with several other gynecologic and non-gynecologic conditions [84,105]. Any debate of diagnosing endometriosis ought to start by defining what endometriosis is, and obtaining a histological confirmatory diagnosis requires surgery which remains the gold standard and at which point the removal of the lesions is unavoidable. This has led to an overall delay of approximately 7 – 12 years from the onset of the symptoms to when a final diagnosis is obtained [46–50], with an approximate 6 of 10 endometriosis cases left undiagnosed [51]. Endometriosis can be suspected based on patient history as well as the symptoms presented. Various studies have tried to enumerate the capability of physical examination in detecting endometriosis by determining its accuracy comparative to surgical diagnosis [210–215]. In these studies, the selection of patients as well as the examination methods differ hence, limiting their comparison for overall estimation of accuracy. In a series of published guidelines in 2014 [216,217], The World Endometriosis Research Foundation (WERF), urged doctors, gynecologists, and researchers alike to homogenize their sample collection methods and data analysis, which were further emphasized as significantly important priorities for endometriosis research in 2016 [218].

Non-invasive methods such as imaging has increasingly become an essential part of the diagnostic process. It has often been used in the investigation of chronic pelvic pain and can be informative in the diagnosis of endometriosis. No imaging technique (described in the following paragraph) or biomarkers have so far been discovered to replace laparoscopy or alone is enough for a confirmed diagnosis of endometriosis.

2.1.3.1 Imaging

Imaging techniques such as ultrasonography have important intrinsic value when identifying the cause of abdominal pain and other than endometriosis, menstrual symptoms. In diagnosing endometriosis, the transvaginal ultrasonography (TVUS), magnetic resonance imaging (MRI) and computer tomography (CT) are some of the techniques of choice used when assessing the presence of endometriosis. These imaging modalities appear to be useful when there is pelvic or adnexal mass [212,219]. When a big mass is found, transvaginal and transabdominal ultrasonography helps to better comprehend the anatomical contribution of organs in close proximity [220]. TVUS is generally the first imaging technique considered when endometriosis is suspected. Guidelines allude to its utility in visualizing the uterine cavity and the endometrium in addition to detecting the characteristic aspects of OMA and DiE or pelvic fluid [83,220–222]. It also has high predictive accuracy in detecting deep

retrocervical and rectosigmoid endometriosis [212,214,223–225]. Hudelist *et al.* reported an increase in the sensitivity of detecting endometriosis when TVUS was combined with pelvic examination compared to when pelvic examination alone was performed [212]. MRI and CT can help characterize pelvic mass as well as discover extra-pelvic foci.

In diagnosing peritoneal endometriosis neither the TVUS or the MRI, has been shown to be effective in detection [226] except when the endometriotic implants are hemorrhagic [227,228]. This reduces the value for early detection in adolescents with endometriosis related symptoms as peritoneal endometriosis is the most predominant form found in this group [229]. In comparison with the other imaging techniques the MRI is less often used for the evaluation of endometriosis owing to associated cost. It is however useful in cases where there are lesion greater than 5mm in size, extensive pelvic adhesion that distort normal pelvic anatomy or suspected ureteral involvement [230] or where ultrasonography findings are ambiguous [83,105]. The MRI has remarkably high diagnostic accuracy for detecting OMA and uterosacral ligaments (USL). OMA on T1-weighted images (T1-WI) with or without fat suppression [231], and USL on thin-sectioned oblique axial T2-weighted images (T2-WI) [232]. Recent studies have suggested that the MRI plays a vital role in detecting deep pelvic endometriosis, especially lesions located in the rectovaginal septum and vagina when integrated with opacity of both the vagina and rectum [233,234]. The MRI holds an advantage over TVUS in that result interpretation is less operator dependent [235]. For each of these imaging techniques to be reliable in diagnosis it requires a radiologist, gynecologist skilled in identifying endometriosis.

2.1.3.2 Diagnostic tools for endometriosis

There have been several attempts at creating noninvasive symptom and imaging based diagnostic tools to help predict the risk of endometriosis before surgery. Fasciani *et al*. established a literature-based Endometriosis Index that encompassed thirty-eight variables as well as parameters obtained from patient pain assessment, consultations, and diagnostic evidence to forecast the existence of endometriosis by location and in general. It relies on a wide-ranging set of diagnostic parameters which includes imaging, laboratory tests, and pelvic examinations, demonstrating its potential use as a non-invasive assessment tool to identify endometriosis and distinguish between the severities of the disease [236]. Yeung *et al.* established a predictive mathematical model to predict early-stage endometriosis with variables from preoperative questionnaire comparable but different from the World Endometriosis Research Foundation-Women's Health Symptom Survey (WERF-WHSS). Their final model comprised of five variables that was able to distinguish between women with and without endometriosis with an area under the curve (AUC)

of 0.822, a sensitivity and specificity of 80.5% and 57.7% respectively as well as a $P < 0.001$; it is however not practicable as a simple self-completed screening tool given the complexity in the scoring system [237]. Forman *et al.* created a 7-point based questionnaire that is centered on symptoms and medical history to distinguish between women with endometriosis and women with a healthy pelvis [238].

Calhaz-Jorge *et al.* created a mathematical model to predict endometriosis founded on symptomology and medical history gathered using a standard questionnaire in subfertile women. In their predictive model dysmenorrhea, oral contraception use, chronic pelvic pain, obesity, and subfertility were factors discovered to be predictive of endometriosis. The authors came to the conclusion that their model might be useful for doctors managing subfertility to help in deciding when laparoscopy should be carried out; however, the research did not rule out previous pelvic surgery for patients and no validation was conducted outside the study population [239]. Eskenazi *et al.* evaluated whether the surgical diagnosis of endometriosis could be predicted through symptoms, pelvic examination, medical history, and ultrasonography discoveries. Both pelvic examination and ultrasonography were effective in predicting ovarian endometriosis. The other non-invasive methods assessed were somewhat moderate in their success but less dependable in predicting non ovarian endometriosis. In the evaluation the presence of any symptom categorized 66% of endometriosis findings (both non-ovarian and ovarian combined), with a less favorable predictive capacity than a positive ultrasound (Cohen's kappa coefficient (κ) of 0.32 vs. 0.58, respectively) [210]. Hackethal *et al.* assessed if when compared with retrospective review of hospital records, a well-defined questionnaire, could enhance the documentation of endometriosis specific variables during preoperative assessment of women with suspected or confirmed endometriosis. They used parameters such as the history of endometriosis, surgical history, fertility/pregnancy, hormonal treatment, family history of endometriosis, menstrual history, allergies, other illnesses, and pain symptoms of endometriosis. The authors reached the conclusion that the use of a well-defined questionnaire did improve the availability of endometriosis specific clinical history in patients with suspected or known endometriosis, although the study made no attempt to distinguish between women with and without endometriosis [240]. Ballard *et al.* explored the usefulness of the different components of chronic pelvic pain in diagnosing endometriosis before laparoscopy. The questionnaire assessed 40 pain descriptors to assess the description of pain, intensity, and location of pain, as well as the observed variations in the proportions of pain between women with and without endometriosis, and also between the women with deep versus peritoneal endometriosis. The identified symptoms in this research could be useful distinguishing between women with and without endometriosis [241]. Nnoaham *et al.* developed a model based on symptoms to predict all the different

forms of endometriosis, in symptomatic women with no prior surgical diagnosis as well as predict stage III/IV endometriosis. The authors used multiple logistic regression analyses, with parameters that include 25 elements from the WERF-WHSS questionnaires; intensity and frequency of pelvic pain; medical, family, and gynecological histories; as well as lifestyle, physical attributes, and demographic characteristics. The models were subsequently validated independently using receiver-operating characteristic curve analysis (ROC). The prediction of endometriosis at any stage was comparatively poor with AUC = 68.3 but was slightly improved with ultrasonographic evidence of ovarian cysts or nodules. The model predicted endometriosis in stages III/IV with comparatively good accuracy AUC = 84.9, with a sensitivity and specificity of 82.3% and 75.8% respectively and also with a cut-off of 0.24 [242].

The Endometriosis Research Center self-evaluation is a 10-item questionnaire to predict endometriosis based on symptoms and medical history. Women who give positive answers to three or more questions are predicted to have endometriosis and encouraged to consult with a doctor to deliberate on a conclusive diagnosis and possible treatment options. And also women who answers positively to three non-symptom questions such as infertility or ectopic pregnancy; autoimmune diseases; history of pelvic surgery, family history of endometriosis; or miscarriage, could be positive for endometriosis [243]. **Table 4** lists studies where various attempts have been made at producing diagnostic tools for endometriosis.

Table 4. List of studies with attempts at producing diagnostic tools for endometriosis.

Study design and population	Type of tool	Method of diagnosis	Parameters assessed	Assessment of performance
Endometriosis studies (general)				
Prospective study of consecutive women with ≥ 2 years of subfertility undergoing laparoscopy and tubal hydrotubation (N = 104) [238]	Patient-completed questionnaire	Laparoscopically visualized endometriosis	Period pain; pelvic pain; dyspareunia; coil; vaginal discharge; laparotomy; nulligravida	Performance and validation not reported
Cross-sectional study. Analysis of survey questionnaire data (N = 26,898) [244]	Patient-completed questionnaire	Endometriosis diagnosis listed in medical records	Severe dysmenorrhea; chronic pelvic pain; dyspareunia; infertility; oral pill as contraceptive	Sensitivity was between 16% and 58%; while specificity was between 70% and 96%
Prospective single-center observational study. Women referred for chronic pain or infertility or with clinical suspicion of endometriosis. (N = 120) [236]	Endometriosis index based on patient evaluation, consultation, and diagnostic evidence. software-assisted scoring using logistic regression	Laparoscopically visualized endometriosis	Predictors of endometriosis based on 38 variables and parameters	Score > 28 test was predictive of deep-infiltrating endometriosis with 72.4% sensitivity and 90.1% specificity
Cross-sectional survey, respondent to online survey (N = 48,020) [73]	Self-report with suspicion or diagnosis of endometriosis	Endometriosis diagnosis or suspicion of endometriosis	Menstrual pelvic pain/cramping; non-menstrual pelvic pain/cramping; dyspareunia; heavy menstrual bleeding; excessive or irregular bleeding; passage of clot; irregular menstrual periods (timing/duration); constipation/bloating/diarrhea; fatigue/weariness/anemia; infertility	Menstrual pelvic pain/cramping (OR: 1.6, 95% CI: 1.4-1.8); non-menstrual pelvic pain/cramping (OR: 4.1, 95% CI: 3.6-4.6); dyspareunia (OR: 3.1, 95% CI: 2.8-3.5); heavy menstrual bleeding (OR: 1.5, 95% CI: 1.3-1.7); excessive or irregular bleeding (OR: 2.1, 95% CI: 1.8-2.4); passage of clot (OR: 1.8, 95% CI: 1.6-2.0); irregular menstrual periods (timing/duration) (OR: 1.5, 95%

				CI: 1.3-1.7); constipation/bloating/diarrhea (OR: 1.9, 95% CI: 1.7-2.2); fatigue/weariness/anemia (OR: 2.2, 95% CI: 2.0-2.5); infertility (OR: 3.6, 95% CI: 3.0-4.4)
Prospective single-center observational study. Women attending a tertiary referral center reporting endometriosis associated chronic pelvic pain > 6 months (N = 90) [237]	Predictive mathematical model for early-stage endometriosis	Laparoscopically visualized and histological confirmed endometriosis	Physical and demographic characteristics; medical and family history; symptom; and quality of life	AUC = 0.822, P < 0.001, Sensitivity = 80.5% and Specificity = 57.7% (cutoff = 0.3091)
Retrospective case-control study involving women who underwent laparoscopy for infertility evaluation (341 with endometriosis; 332 with normal pelvis) [245]	Predictive mathematical model	Laparoscopically visualized endometriosis	Family history of endometriosis; history of galactorrhea; history of pelvic surgery; dysmenorrhea; pelvic pain; dyspareunia; premenstrual spotting; fatigue	Family history of endometriosis (OR: 2.7, 95% CI: 1.06-7.1); history of galactorrhea (OR: 1.8, 95% CI: 1.1-3.05); history of pelvic surgery (OR: 14.5, 95% CI: 6.1-34.2); dysmenorrhea (OR: 1.8, 95% CI: 1.1-2.8); pelvic pain (OR: 4.1, 95% CI: 2.4-6.8); dyspareunia (OR: 1.6, 95% CI: 1.09-2.4); premenstrual spotting (OR: 2.2, 95% CI: 1.3-3.6); fatigue (OR: 2.6, 95% CI: 1.3-5.1)
Prospective study (study sample) (N = 90); Retrospective record review (test sample) (N = 120) [210]	Patient evaluation and noninvasive diagnostic procedures	Laparoscopically visualized endometriosis	No endometriosis; nonovarian endometriosis; ovarian endometriosis	Presence of symptoms correctly classified 66% of diagnosis. Validation not reported
Prospective, observational study in a referral unit; women who underwent laparoscopy for chronic pelvic pain (N = 144) [246]	Patient evaluation and noninvasive diagnostic procedures	Laparoscopically visualized endometriosis	Noncyclical pain; dysmenorrhea; dyspareunia; dyschezia	Noncyclical pain (Endometriosis, 62.5%; no endometriosis, 70.8%; *p* = 0.48); dysmenorrhea (Endometriosis, 79.1%; no

				endometriosis, 87.5%; p = 0.37); dyspareunia (Endometriosis, 25.0%; no endometriosis, 33.3%; p = 0.46); dyschezia (Endometriosis, 25.0%; no endometriosis, 20.8%; p = 0.69)
Retrospective study of sub fertile women undergoing diagnostic or therapeutic laparoscopy (N = 1079) [239]	Predictive mathematical model	Laparoscopically visualized endometriosis	Primary subfertility; dysmenorrhea; chronic pelvic pain; oral contraception; obesity (inverse relationship)	Multivariate prediction model AUROC = 0.71 for all endometriosis and 0.74 for grade III/IV endometriosis validation not reported
Prospective multi-center observational study. Operative cohort from ENDO study – Women without a history of surgically confirmed endometriosis who underwent laparoscopy or laparotomy (N = 473) [74]	Patient evaluation and noninvasive diagnostic procedures	Surgically visualized endometriosis	Chronic pelvic pain; cyclic pelvic pain; vaginal pain with intercourse; deep pain with intercourse; burning vaginal pain after intercourse; pain just before menstrual period; level of cramps with period; pain after period is over; pain at ovulation (mid-cycle); dysuria; dyschezia	Chronic pelvic pain (Endometriosis, 44.2%; other, 39.0%; normal pelvis, 30.2%; p = 0.04); cyclic pelvic pain (Endometriosis, 49.5%; other, 31.0%; normal pelvis, 33.1%; p < 0.001); vaginal pain with intercourse (Endometriosis, 54.7%; other, 41.5%; normal pelvis, 32.4%; p < 0.001); deep pain with intercourse (Endometriosis, 53.2%; other, 38.1%; normal pelvis, 30.9%; p < 0.001); burning vaginal pain after intercourse (Endometriosis, 33.2%; other, 22.5%; normal pelvis, 22.1%; p = 0.03); pain just before menstrual period (Endometriosis, 75.3%; other, 61.9%; normal pelvis, 66.2%; p = 0.03); level of cramps with period (Endometriosis, 91.1%; other, 85.0%; normal pelvis, 79.4%; p = 0.01); pain after

				period is over (Endometriosis, 38.4%; other, 26.5%; normal pelvis, 38.2%; $p = 0.04$); pain at ovulation (mid-cycle) (Endometriosis, 67.4%; other, 49.0%; normal pelvis, 52.2%; $p = 0.001$); dysuria (Endometriosis, 22.6%; other, 19.1%; normal pelvis, 11.0%; $p = 0.03$); dyschezia (Endometriosis, 44.2%; other, 32.7%; normal pelvis, 25.7%; $p = 0.002$)
Comparative study of women undergoing laparoscopy for chronic pelvic pain (N = 185) [241]	Patient-completed questionnaire	Laparoscopically visualized endometriosis	40 pain descriptors for three different aspects of pain: 1. Descriptions of pain, 2. Anatomical arears of pain, and 3. Intensity of pain	Performance not reported
Retrospective cohort study of women with or without pelvic pain eveluated for infertility (N = 80) [247]	Predictive mathematical model	Histologically verified endometriosis	Premenstrual spotting for ≥2 days; dysmenorrhea; dyspareunia	Premenstrual spotting for ≥2 days (Sensitivity, 76%; specificity, 90%; PPV, 96%; NPV, 74%; accuracy, 81%); dysmenorrhea (Sensitivity, 87%; specificity, 63%; PPV, 75%; NPV, 79%; accuracy, 76%); dyspareunia (Sensitivity, 38%; specificity, 83%; PPV, 74%; NPV, 51%; accuracy, 58%)
Retrospective review of hospital records for women presenting with suspected or known endometriosis (N = 69) [240]	Patient-completed questionniare	Surgically visalized endometriosis	34-item questionniare about the histroy of endometriosis; surgical histroy; allergies and other illnesses; family history; fertility/pregnancy; hormone treatment; menstrual history; and visual analog scales for common painful symptoms of endometriosis	Performance not reported

ENDO Study – prospective, matched-exposure chort study comprising women undergoing pelvic surgery (N = 495) and a matched cohort (N = 131) [75]	Predictive mathematical model	Surgically visualized endometriossis (operative cohort) Pelvic MRI-diagnosed endometriosis (matched cohort)	History of infertility; dysmenorrhea; pelvic pain; pelvic pain (surgical indication)	History of infertility (OR: 2.43, 95% CI: 1.57-3.76) [operative]; 7.91 (1.69-37.2) [matched]; dysmenorrhea (OR: 2.46, 95% CI: 1.28-4.72) [operative]; 1.41 (0.28-7.14) [matched]; pelvic pain (OR: 1.39, 95% CI: 0.95-2.04) [operative]; 0.76 (0.09-6.54) [matched]; pelvic pain (surgical indication) (OR: 3.67, 95% CI: 2.44-5.50) [operative]
Prospective, multi-center observational study of symptomatic women with scheduled laparoscopy (N = 1396) [242]	Predictive symptom-based model	Laparoscopically visualized endometriosis	Mutiple factors such as dysmenorrhea; dyschezia; nonmenstrual pelvic pain; ovarian cyst; family history; race; etc. Model and ultrasound	Factors (Sensitivity, 85%, specificity, 44%); Model and ultrasound (Sensitivity, 58%; specificity, 89%) Area under ROC curve = 0.683
Prospective cohort of women with chronic pelvic pain (N = 284) [248]	Patient evaluation and noninvasive diagnostic procedures	Laparoscopically or histologically confirmed endometriosis	Anterior vaginal wall tenderness (endometriosis and other pathology); Anterior vagnal wall tenderness (endometriosis only)	Anterior vaginal wall tenderness (endometriosis and other pathology) Sensitivity, 93%; Anterior vagnal wall tenderness (endometriosis only) Sensitivity, 17%
Retroscpective cohort of women evaluated for chrronic pelvic pain (N = 331) [249]		Histologically verified endometriosis	Short-form *MPQ* pain descriptor: Cramping; Sickening; Tiring/exhausting; Shooting; Punishing/cruel; Splitting	Cramping days (Sensitivity, 92%; specificity, 33%; PPV, 40%; NPV, 89%); Sickening (Sensitivity, 73%; specificity, 46%; PPV, 40%; NPV, 78%); Tiring/exhausting (Sensitivity, 77%; specificity, 38%; PPV, 38%; NPV, 77%); Shooting (Sensitivity, 70%; specificity, 43%; PPV, 37%; NPV, 75%); Punishing/cruel (Sensitivity, 49%; specificity, 65%; PPV, 40%; NPV, 72%); Splitting (Sensitivity, 36%; specificity, 77%; PPV, 43%; NPV, 71%)

Prospective study of consecutive women with unexplained infertility (N = 55) [250]	Patient evaluation and noninvasive diagnostic procedures	Laparoscopically or histologically confirmed endometriosis	Anterior vaginal wall tenderness	Sensitivity, 84%; specificity, 75%; PPV, 86%; NPV, 69%
Retrospective case series compaising infertile women with regular cycles and no prior endometriosis diagnosis (N = 221) [78]	Predictive mathematical model	Histologically verified endometriosis	Pelvic pain; pelvic pain and type of infertiltity, age, and duration of infertility	Pelvic pain (Sensitivity, 59%; specificity, 56%; PPV, 54%; NPV, 57%); pelvic pain and type of infertiltity, age, and duration of infertility (Sensitivity, 65%; specificity, 73%)
Prospective study of consecutive women with symptoms of endometriosis (N = 200) [212]		Histologically verified endometriosis	Vaginal examination; vaginal examination and TVS	Vaginal examination (Sensitivity, 23-88%; specificity, 89-100%; PPV, 65-100%; NPV, 85-99%; accuracy, 86-99%); vaginal examination and TVS (Sensitivity, 67-100%; specificity, 86-100%; PPV, 50-100%; NPV, 93-100%; accuracy, 86-100%)
Respondent to a self-adminstered questionnaire (N = 1285) [251]	Patient-completed questionniare	Self reporeted surgically confirmed endometriosis	Dymenorrhea; severe dysmenorrhea; dyspareunia; problems conceiving; chronic pelvic pain	Dymenorrhea (Cases, 82.5%; general population, 59.3%; $P < 0.001$); severe dysmenorrhea (Cases, 65.9%; general population, 52.9%; P = NS); dyspareunia (Cases, 52.0%; general population, 20.0%; $P < 0.001$); problems conceiving (Cases, 70.6%; general population, 25.2%; $P < 0.001$); chronic pelvic pain (Cases, 80.0%; general population, 22.9%; $P < 0.001$)

Multicenter National case-control study comprising women with endometriosis (N = 5540) and matched controls (N = 21,239) [41]	Diagnostic model based on symptom collected via patient completed questionnaire	Diagnostic or procedural codes consistent with endometriosis recorded in a nationawide general practice databae	Dsymenorrhea; pelvic pain; dyspareunia; abdminal pain; menorrhagia; intermenstrual pain; infertility/subfertility; plevic inflammatory disease; ovarian cysts; ovary pain	Dsymenorrhea (OR: 9.8, 95% CI: 8.8-10.9); pelvic pain (OR: 13.5, 95% CI: 11.7-15.7); dyspareunia (OR: 9.4, 95% CI: 8.0-11.1); abdminal pain (OR: 5.9, 95% CI: 5.5-6.4); menorrhagia (OR: 5.0, 95% CI: 4.6-5.5); intermenstrual pain (OR: 6.9, 95% CI: 4.7-10.2); infertility/subfertility (OR: 6.2, 95% CI: 5.4-7.1); plevic inflammatory disease (OR: 6.4, 95% CI: 5.6-7.4); ovarian cysts (OR: 12.2, 95% CI: 9.9-15.0); ovary pain (OR: 9.1, 95% CI: 3.2-26.0)
Site-specific endometriosis studies				
Retroscpective, comparative study of women reffered for investigation and treatment of endometriosis undergoing subsequent laparoscopy (N = 51) [252]	Retrospective, observational analysis of patient-reported sysmptoms	Laparoscopically visualized endometriosis	Dysmenorrhea; dyspareunia; infertility; dyschezia; rectal pain; cyclical and noncycical rectal bleeding; tenesmus; apareunia; nausea; abdominal bloating and diarrhea	Apareunia and nausea or abdominal bloating were strong markers for rectovaginal disease with a prevalence of 87 and 89% respectively. Validation not reported
Retrospective analysis of consecutive women with ovarian endometrioma who underwent surgery (N = 178) [253]	Predictive mathematical model to predict DIE in patients with ovarian endometrioma	Histologically verified endometriosis	Model includes previous preganacy; history of surgery for endometriosis; endometriosis-associated pelvic pain score	Sensitivity, 80%; specificity, 84%
Retrospective study of women scheduled for laparoscopy for chronic pelvic pain symptoms (N = 134) [67]	Diagnostic model based on symptom collected via self administered questionnaire	Surgically visalized endometriosis	Dysmenorrhea; dyspareunia; nonmenstrual pain; and urinary or gastrointestinal symptoms during menstruation	Area under the ROC curve, 0.77; sensitivity, 74.5%; specificity, 68.7%; positive likelihood ratio, 2.4; negative likelihood ratio, 0.4; validation not reported

Prospective, single-center study of women with a histological diagnosis of endometriosis (N = 211) [254]	DIE score calaculated from multiple regression model from preoperative symptom questionnaire	Histologically verified endometriosis	Infertility (primary or secondary); duration of pain > 24 mo; VAS deep dyspareunia > 5; VAS GI symptoms ≥ 5; severe dysmenorrhea	Infertility (primary or secondary) (Sensitivity, 51%; specificity, 73%; OR, 1.5; *P* = 0.003); duration of pain > 24 mo (Sensitivity, 62%; specificity, 81%; OR, 7.1; *P* < 0.001); VAS deep dyspareunia > 5 (Sensitivity, 69%; specificity, 59%; OR, 3.2; *P* = 0.007); VAS GI symptoms ≥ 5 (Sensitivity, 75%; specificity, 76%; OR, 9.3; *P* < 0.001); severe dysmenorrhea (Sensitivity, 55%; specificity, 75%; OR, 3.5; *P* < 0.001)
Retrospective and prospective study of women undergoing surgery for DIE (N = 46) [255]	Assessment of clinical signs and anataomic sites using Lasmar map [256]	Surgically visalized endometriosis	Assessment of anatomical areas affected by endometriosis using site of disease recorded by medical history; physical examination immaging tests without laparoscopy; age; parity; skin color; Symptom such as dysmenorrhea; hypermenorrhea; pelvic pain; not related to menstrual cycle, dypareunia; dyschezia; or uninary symptoms	Preoperative clincal evaluation / Lasmar map had high sensitivity, specificity, and accuracy. Validation not reported
Retrospective, longitudinal study of consecutive women with clincal evidence of endometriosis (N = 92) [213]	Accuracy of physical examination, transvaginal sonography, rectal endoscopic sonography, and magnetic resonance imaging	Laparoscopically visalized endometriosis	Vaginal examination; TVS; Rectal endoscopic sonography; MRI	Vaginal examination (Sensitivity,18-74%; specificity, 72-96%; PPV, 40-97%; NPV, 24-90%; accuracy, 54-87%); TVS (Sensitivity,9-94%; specificity, 67-100%; PPV, 50-100%; NPV, 25-89%; accuracy, 77-96%); Rectal endoscopic sonography (Sensitivity,7-89%; specificity, 44-100%; PPV, 33-100%;

			NPV, 9-90%; accuracy, 48-90%); MRI (Sensitivity,55-87%; specificity, 86-99%; PPV, 73-99%; NPV, 38-94%; accuracy, 84-94%)

AUC, area under the curve; *CI*, confidence interval; *DIE*, deep-infiltrating endometriosis; *ENDO*, Endometriosis: Natural History, Diagnosis, and Outcomes Study; *GI*, gastrointestinal; *HR,* hazard ratio; *MPQ*, McGill Pain Questionnaire; *MRI*, magnetic resonance imaging; *NPV*, negative predictive value; *PPV*, positive predictive value; *ROC*, receiver-operating characteristic; *TVS*, transvaginal sonography; *VAS*, visual analogue scale.

2.1.3.3 Surgical diagnosis

Surgery and histological confirmation remains fundamental tools in diagnosing endometriosis [83,105,221]. It makes the direct visualization and identification of the disease possible. Histological confirmation is strongly recommended as a result of the minimal reliability of visual confirmation alone, as endometriosis is frequently not discovered amongst a quarter of women who undergo laparoscopy for chronic pelvic pain [257–260]. The peritoneal lesions are probably the most challenging to diagnose surgically owing to their visual appearance being heterogeneous, while at the same time the unpigmented peritoneal lesions are highly active endometriosis implants [69]. Typically, the histological appearance of endometriosis implants consists of endometrial glands, stroma, and hemosiderin laden macrophages.

2.1.4 Biomarkers for endometriosis diagnostics

The quest for a biomarker for endometriosis has been a challenging and ongoing issue and decades of scientific studies have not resulted in a reliable biomarker for the non-invasive diagnosis or prognosis of endometriosis. One of the reason is that, as a heterogeneous disease, the different forms of endometriosis could express various markers differently [261]. However, an array of potential molecular markers have been identified [262–265]. Biomarker investigations have included serum or plasma, menstrual blood, peritoneal fluid, urine and endometrial tissue [266]. As a chronic inflammatory disease, hormones, cytokines, angiogenic factors, growth factors and chemokines, all play a part as etiological factors in endometriosis. While no reliable biomarker has been identified among these factors, having a non-invasive marker would be a valuable early detection tool in symptomatic women with relatively normal outcomes on pelvic ultrasonography and at best such biomarker must be relevant in diagnosing all disease stages (particularly minimal to mild endometriosis) with relatively high sensitivity [267], independent of menstrual cycle phase. Despite The Fact That around a 100 putative biomarkers have been proposed for endometriosis, recent systematic reviews of serum and endometrial biomarkers found none that demonstrated clear evidence to support their diagnostic role or use in clinical settings [268–270]. Most of the studies have been relatively small without sufficient positive and negative control groups, and some are considered to have poor methodological quality. Endometriosis flourishes in an estrogen dominant environment, hence hormonal markers such as progesterone, estradiol, luteinizing hormone (LH), activin and leptin, have all been studied but none has been shown to be useful as a serum marker [270]. A recent meta-analysis study evaluated seven different hormone markers in the serum of 1279 women with endometriosis in the 17 studies in the meta-analysis a pooled sensitivity and specificity of 79% and 89% respectively was reported for aromatase and concluding it to be a potentially good

diagnostic test. They nevertheless, recognized that their findings was based on modest quality research [271].

2.1.4.1 Serum Biomarker

Among serum markers for endometriosis, cancer antigen 125 (CA-125) a glycoprotein that is encoded by the MUC16 gene [272,273] is the most studied. CA-125 is expressed in some derivatives of the coelomic epithelium [274,275], and is known to be elevated in the serum of patients with certain types of cancers [275–278]. It is also been reported to be elevated in other malignant and benign diseases [279–282]. In advanced endometriosis CA-125 is reported to be elevated (>35 U/ml) with a sensitivity and specificity of 61.1% and 87.5% respectively. However, disappointingly it shows only moderate sensitivity and specificity for minimal and mild endometriosis [283–291]. In a systematic review and meta-analysis of 14 studies, CA-125 was reported to have a cut-off level of ≥30 U/ml with a sensitivity and specificity of 93% and 52% respectively [281]. However, according to Santulli *et al.* the CA-125 mean serum level in OMA = 60.8 U/ml, in DIE = 55.2 U/ml and in peritoneal endometriosis = 23.2 U/ml [292] are indicative for endometriosis. During the menstrual cycle CA-125 oscillates and levels are relatively high in both endometriosis patients and healthy women [293,294]. These inadequacies make CA-125 an unreliable biochemical test for endometriosis diagnosis [281,292]. Another ovarian tumor marker investigated for endometriosis is CA-19-9 which was originally discovered in patients with colorectal carcinoma. It was shown to be relatively high in endometriosis and has an analogous or lower sensitivity than CA-125 [268]. However, the results are conflicting, as other studies have been unsuccessful in finding any connections between endometriosis and CA-19-9 serum levels [295], consequently, demonstrating an unsatisfactory diagnostic role in clinical settings.

Other glycoproteins such as alpha-fetoprotein (AFP), CA-15-3, CA-72, β-2 microglobulin, carcinoembryonic antigen, haptoglobin and follistatin have all been investigated over the years. Haptoglobin β chain isoforms levels have been shown to be substantially higher in the serum of women with endometriosis than in controls [296]. Follistatin, an activin A inhibitor, encoded by the *FST* gene [297,298] is richly expressed in the endometriotic tissues and also in the endometrium in all menstrual cycle phases [299]. Recent studies have demonstrated a significant increase in serum follistatin levels in women with endometriosis [268] particularly in patient with OMA when compared with controls, with a sensitivity of 92% and a specificity of 92% at 1433 pg/ml cut-off [300] but with unreproducible results [301].

2.1.4.2 Inflammatory cytokines and immunological markers

Inflammatory and immunological factors are considered to be involved in the development of endometriosis. Therefore, its role has been extensively examined in women with surgically verified endometriosis and compared to healthy controls as possible biomarkers for endometriosis [266,268,269]. In the search for a noninvasive diagnosis of endometriosis a number of cytokines have been evaluated, that includes interleukins IL-1, IL-6, IL-8, Tumor necrosis factor alpha (TNF-*α)*, Monocyte chemoattractant protein-1 (MCP-1), and interferon-*γ* (IFN-*γ*) [268]. There is very limited data and presently none of the evaluated inflammatory cytokines or chemokines have proven useful as a biomarker [38,269,302].

IL-6 and TNF-α are some of the most investigated for endometriosis of all cytokines [303]. IL-6 is a pro-inflammatory cytokine deemed to play an essential role in the growth and maintenance of ectopic endometrial tissues. It is involved in the activation of T-cell, differentiation of B-cells (B lymphocytes) and in the secretion of other cytokines and in patients with endometriosis its expression has been shown to be dysregulated in macrophages. Some studies have demonstrated increased levels of IL-6 in the serum of endometriosis patients, while other studies have not been able to replicate the result using different cut-off values. In one of such studies the levels of IL-6 in the serum of women with minimal to mild endometriosis was higher than in other groups. The authors reported a significant accuracy for IL-6 in detecting endometriosis using a cut-off level of 25.75 pg/ml, and reported a sensitivity and specificity of 75.0% and 83.3% respectively, suggesting IL-6 to be a reliable, marker for minimal and mild endometriosis [304]. In another study, in infertile patients with endometriosis the values of T-helper pathway related interleukins such as IL-10, IL-12, IL-17, and IL-23 were comparable to healthy controls with infertility [305]. Neither did the evaluation of other serum cytokines, such as IL-2, IL-4, IL-18, IL-13, IL-15, IL-16 produce any additional correlation with endometriosis [303].

TNF-α is secreted from activated macrophages, and it plays a pro-inflammatory and pro-angiogenic role in the endometrium where it is responsible for shedding and proliferation [303,306]. In women with endometriosis TNF-α is elevated in peritoneal fluid [307], with some studies showing increased levels correlating with severity of the disease [308]. However, serum TNF-α findings remain inconclusive as some studies have reported elevated levels of TNF-α in women with endometriosis [309], while other studies show no difference between endometriosis and control women [264].

In some studies the inflammatory marker C-reactive protein (CRP) was shown to be significantly increased in women with endometriosis when examined with high sensitivity assays [310]. However in other studies the upregulation could not be determined [268,311].

2.1.4.3 Endometrial biomarkers

Like with serum biomarkers numerous tissue biomarkers have been proposed for the diagnosis of endometriosis. Although more invasive than serology, apart from the minimal irritation to the patient, endometrial biopsies could be beneficial not just to test the receptivity of the endometrium in infertile women with or without endometriosis [312], but could also offer a prospective advantage for improved specificity as the eutopic endometrium demonstrates aberrant extraordinary sex steroid driven cyclic alteration and regenerative ability and can be easily obtained without the need for anesthesia. Genome-wide profiling of the normal endometrium showed enormous variations in the molecular composition between samples taken from all the phases of the menstrual cycle [313]. The generally accepted hypothesis of retrograde menstruation [314] implies that the menstrual endometrium is the origin of the ectopic endometriosis lesions. Therefore, using the endometrium is perhaps a sensible approach in the pursuit to identify biomarkers for endometriosis instead of relying on blood or even urine for prospective biomarkers, which could encompass other relevant information than just specifying the existence of endometriosis in women.

A Cochrane review of 54 diagnostic studies [270] assessed endometrial biomarkers in either specific menstrual cycle phases or outside of it. The studies analyzed the biomarkers both in the menstrual fluid, as well as the entire endometrial tissue or in separate endometrial components. In some of these studies a variety of endometrial biomarkers that included inflammatory markers (such as IL-1R2), angiogenesis and growth factors (such as Prokineticin, PROK-1), neural markers (such as CGRP, NF, NPY, PGP 9.5, SP, VIP), DNA-repair molecules (such as human telomerase reverse transcriptase, hTERT), endometrial and mitochondrial proteome, cell adhesion molecules (such as integrins α3β1, α4β1, α6 and β1), hormonal markers (such as 17βHSD2 (17β-Hydroxysteroid dehydrogenase 2), CYP19A1, ER-α, ER-β), myogenic markers (such as caldesmon, CALD-1), and tumor markers (such as CA-125) were evaluated for their diagnostic performance. Many of the above-mentioned biomarkers were evaluated in single studies, and only PGP 9.5 and CYP19A1 were reported to demonstrate significant diversity for a diagnostic marker. However, there were too limited data to reliably determine any source of heterogeneity. In another study by Naqvi *et al.* on the extent of DNA methylation in the endometrium of women with endometriosis five hypermethylated (CDCA2, DUSP22, MGMT, ID2, and RBBP7) and five hypomethylated (BMPR1B, IGSF21, TNFRSF1B, TP73, and ZNF681) genes were compared in the eutopic endometrium of patients versus healthy controls and no significant differences were found when compared with previously reported genes associated with the pathogenesis of endometriosis, which includes ER-β, CYP19A1 (aromatase), COX-2, PR-B, and SF1 [315]. When considering endometrial biopsies to diagnose endometriosis, the archetypal

progesterone resistance of eutopic endometrium where the unresponsiveness to progesterone results in an incomplete transition of the endometrium from the proliferative to early secretory phase of menstrual cycle in women with moderate to severe endometriosis, is just as important [38,316]. Knowing this, we can deduce that through identifying the impairment of steroid dependent genes as compared with healthy control population could help theoretically diagnose endometriosis from endometrial biopsies [38].

2.1.5 Omics -analyses on endometriosis

The phrase "**omics**" when added to a molecular term suggests a comprehensive assessment of a set of molecules [317]. It references a field of study in biological sciences that ends with the suffix -*omics*, such as genomics, transcriptomics, proteomics, metabolomics, etc. (**Figure 10**). The advent of cost-effective, high-throughput technologies has transformed medical research and in the past decade genotyping arrays and current new generation sequencing technologies (NGS), combined with the development of high quality human genome reference maps, comprehensive statistical tools, and large harmonized cohorts of thousands of patients, has enabled genome-wide association studies (GWAS) and methods for examining global transcript levels [317–320]. The capability to analyze global gene expression patterns rapidly found its implementation in many fields of natural science, including the analysis of disease. These novel technologies also made mapping the loci that control gene expression a possibility, referred to as the expression quantitative trait loci (eQTL), which have been proven valuable in interpreting GWAS and the development of biological networks [317]. Genomics was the first of the omics disciplines developed, more specifically it focused on the science of the structure, function, evolution, and mapping of genomes. It delivered a valuable framework for the characterization and quantification of exact genetic variants contributing equally to hereditary and multifaceted diseases as opposed to "genetics" that studies individual variants of a single genes. As the ability to identify genetic variants that are linked to complex diseases increased so too did the realization that shaped ensuing approaches to explaining the cause of disease.

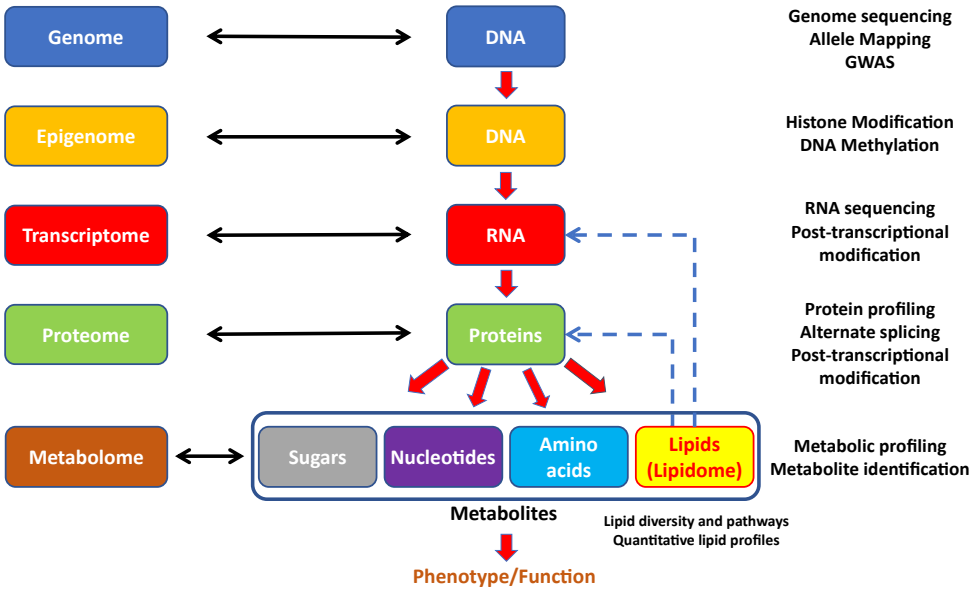


Figure 10. *Major omics and how they relate to each other*. Systems biology 'omics' technologies i.e., genomics, epigenomics, transcriptomics, proteomics, and metabolomics enable high-throughput quantitative profiling of molecules in biological systems. Image modified from *Wikipedia. (Image licensed under the Creative Commons Attribution-ShareAlike 3.0 License).*

All identified loci thus far describe just a portion of the inherited elements for certain diseases, although hereditary diseases usually stem from alterations in the genes coding regions, common disease generally results from alterations in the regulation of genes. All these realizations provide a justification for the advancement of technologies involved in integrating various omics data types to recognize molecular patterns that are linked to diseases [317]. Omics technologies can identify and evaluate hundreds of markers and each omics data type usually provides a list of associated differences with the disease. These may inform the understanding to the distinctions between the disease and control groups and may be useful as disease process markers.

Innovations in advanced technologies and computational biology have resulted in the generation of large biological datasets which in turn has increased methodologies for the use of omics in endometriosis research. Omics has also allowed for big scale molecular assessments of tissues and cells in different conditions. This might be especially useful when studying complicated diseases with unknown pathogenesis, such as endometriosis [321]. In endometriosis study, this has led to a surge in studies aiming to decipher the molecular signatures involved in the disease. Omics methods used in endometriosis research so far, include genomics (variability in DNA sequence in the genome), epigenomics (epigenetic

modifications of DNA), metabolomics (variability in composition and abundance of metabolites), lipidomics (lipid profile within a cell, tissue, organism, or ecosystem), microbiomics (variability in composition and abundance of the microbiota), transcriptomic (variability in composition and abundance of mRNA and miRNA levels) and proteomic (variability in composition and abundance of the proteins) analysis of blood [322], endometrial fluid [323], and tissues (list of omics studies in endometriosis listed in **Table 5**). All which have already been utilized to understand the etiology of endometriosis or support proposed pathogenesis theories and evaluate the severity of the disease. Secretomics a sub-field of proteomics that represents a powerful approach for characterizing and quantifying secretomes- all proteins produced by a cell, tissue, or organism at any particular time or under certain conditions [324] and interactomics which is the study of complex relationships as well as of the effects of these interaction between the proteins as well as other molecules of a cell [325] are two omics methods with no endometriosis related data till date. The biggest benefit of 'omics' studies is that the data can be collected with no existing hypotheses, and fundamental research questions are not necessarily needed (first experiment-then-hypothesis approach) [326].

Table 5. 'Omics' studies in endometriosis. Modified from Saare *et. al* 2017 [321].

	Patients (n)	Controls (n)	Main findings	Reference
Endometrium				
Genome studies	21	9	Gains: +3p, +10q, +13q; losses: −1p, −3p, −4p, −22q; 724 mutated genes	327,328
Epigenome studies	55	46	No common genes	315,329,330
Transcriptome studies	330	203	Differences in PI3K/AKT, JAK/STAT, SPK/JNK, and MAPK, p53, adherens junction, calcium signaling, EGF/PGF/DGF, endothelial biology, protein synthesis, cell division, integrin-mediated cell adhesion, RAS/RAF signaling, decidualization, cellular adhesion, cytokine-cytokine receptor interaction, apoptosis, complement pathway	312,316,331–346
Proteome studies	100	97	Vimentin, peroxiredoxin, HSP70, HSP90, annexins, actins, and 14-3-3 family proteins	332,347–355

Lesions				
Genome studies	130	9	Frequent SCNAs: Gains: 1p, 3p, 6q, 17q, and Xq; Losses: 1p, 5p, and 6q	356–362
EPIGENOME STUDIES	24	27	*HOXD10*	363–365
Transcriptome studies	281	96	Differences in expression of genes involved in organ development; metabolism; action of prostaglandins and glucocorticoids; complement, RAS, MAPK, and PI3K signaling; cytokine-cytokine receptor interaction; cellular adhesion; immune cell recruitment; apoptosis; cell signaling; T-cell cytotoxicity and regulation of inflammatory responses pathways; miR-200 family (epithelial-mesenchymal transition)	331,337,345,346,362,366–386
Proteome studies	35	19	Glycolysis and oxidative respiration, transforming growth factor β-1, calponin-1, and emilin-1, SM-22α and Rab37, Rho-GDIα, haptoglobin, transgelin, smooth muscle actin-binding protein	387–391
Blood and body fluids				
Genome studies	14688 2226	161694 18024	11 significant SNPs 9 CNVs	392–398
Transcriptome studies	79	69	No common miRNAs, 12 miRNAs reported in at least two studies	399–404
Proteome studies	1970	1104	Serum/plasma: HP and A1BG; PF: α1-antitrypsin, α-1b-glycoprotein, S100-A8, serotransferrin, acute phase proteins (haptoglobin and SERPINA1); Menstrual blood: RMP2, UCH-L1, MYL9; Urine: cytokeratin-19, VDBP; EF: proteins involved in cell signaling, cell death, and cell movement processes.	323,352,353,404–428
Metabolome studies	119	114	SMOH (C16:1), ratio (PCaa C36:2/PCae C34:2), 2-methoxyestradiol, 2-methoxyestrone, dehydroepiandrosterone, androstenedione, and cholesterol	429–432

PF peritoneal fluid, *EF* endometrial fluid.

2.1.5.1 Genomics of endometriosis

Genomics is by far the most mature of the omics technologies. It is the study of the whole genetic makeup of an organism known as genomes, as well as the cumulative number of genes in these genomes. In the field of medical research, genomic studies focuses on finding or detecting genetic variants that may be linked with the development of complex diseases, the response to treatment, and the prognosis of the future condition of a patient. GWAS has been successfully applied for the identification of thousands of genetic variants linked with more complex diseases (e.g., GWAS catalog https://www.ebi.ac.uk/gwas/home) in different human populations [317]. GWAS studies offer a substantial contribution to our understanding of complex phenotypes in combination with high throughput technologies, which includes NGS for whole genome (WGS) [433,434], genotype arrays [435–438], and whole exome sequencing (WES) [439] have made significant contributions to the revolution of medical research [434,437].

Despite considerable progress in understanding its pathophysiology, the etiology of endometriosis remains unknown. Several theories have suggested a link between the interactions of various environmental and genetic factors, in the development of endometriosis, with each factor having a causal effect on the risk of developing the disease. GWAS takes candidate genes and single nucleotide polymorphisms (SNPs) into consideration when seeking to untangle the relationship between genomic variation and the development of a disease. Several gene association studies so far conducted, have been performed by examining hypotheses based candidate genes, with the overwhelming majority of them not generating results that can be replicated [440]. Hence, a wide range of nucleotide polymorphism located in the angiogenesis-, autoimmunity-, inflammation-, hormonal function-, proliferation, cellular cycle-, and apoptosis-related loci as well as tumor growth/suppression and detoxification genes have been associated with endometriosis [317]. See (Vassilopoulou, L. *et al.* 2019) [441] for review. Many of these disease related loci discovered through GWAS and *meta*-analyses, are involved in the cell cycle regulation and transcription, in cell adhesion, inflammation, and signaling and also in metabolism and oxidative stress [442–445]. So far, only a handful of genome-wide association studies has been published [206] but they identify 19 different disease associated signals held at 14 different loci **Table 6** [443]. Interestingly, the indicators for most of these loci are clear and distinct at advanced stages (stage III/IV) of endometriosis [10,197,443].

Table 6. GWAS meta-analyses identifying 14 significant loci in endometriosis. Modified from Sapkota *et.al* 2017 [443].

Chr	SNP	Position (bp)	RA	OA	Meta-analysis (All)				Meta-analysis (Grade B)		Associated gene/cytoband
					RAF_{EUR}	RAF_{JPT}	OR (95% CI)	*P* value	OR (95% CI)	*P* value	
Previously reported loci											
1	rs12037376	22462111	A	G	0.17	0.58	1.16 (1.12–1.19)	8.87×10^{-17}	1.28 (1.18–1.36)	2.69×10^{-9}	WNT4/1p36.12
2	rs11674184	11721535	T	G	0.61	0.54	1.13 (1.10–1.15)	2.67×10^{-17}	1.18 (1.10–1.24)	1.94×10^{-6}	GREB1/2p25.1
2	rs6546324	67856490	A	C	0.31	0.21	1.08 (1.05–1.11)	3.01×10^{-8}	1.19 (1.11–1.26)	3.71×10^{-7}	ETAA1/2p14
2	rs10167914	113563361	G	A	0.30	0.75	1.12 (1.08–1.15)	1.10×10^{-9}	1.15 (1.07–1.21)	7.59×10^{-5}	IL1A/2q13
4	rs1903068	56008477	A	G	0.68	0.88	1.11 (1.07–1.13)	1.04×10^{-11}	1.33 (1.24–1.40)	2.58×10^{-15}	KDR/4q12
6	rs760794	19790560	T	C	0.43	0.71	1.09 (1.06–1.12)	1.79×10^{-10}	1.17 (1.10–1.24)	8.74×10^{-7}	ID4/6p22.3
7	rs12700667	25901639	A	G	0.74	0.20	1.10 (1.07–1.13)	9.08×10^{-10}	1.28 (1.19–1.36)	6.69×10^{-11}	7p15.2
9	rs1537377	22169700	C	T	0.40	0.39	1.09 (1.06–1.12)	1.33×10^{-10}	1.21 (1.13–1.27)	6.31×10^{-9}	CDKN2B-AS1/9p21.3
12	rs4762326	95668951	T	C	0.47	0.48	1.08 (1.05–1.11)	2.20×10^{-9}	1.15 (1.08–1.21)	1.08×10^{-5}	VEZT/12q22
Novel loci											
2	rs1250241	216295312	T	A	0.29	0.06	1.06 (1.03–1.09)	6.20×10^{-5}	1.23 (1.15–1.30)	2.99×10^{-9}	FN1/2q35
6	rs1971256	151816011	C	T	0.20	0.35	1.09 (1.06–1.13)	3.74×10^{-8}	1.28 (1.19–1.36)	1.50×10^{-10}	CCDC170/6q25.1
6	rs71575922	152554014	G	C	0.16	-	1.11 (1.07–1.15)	2.02×10^{-8}	1.35 (1.24–1.43)	2.87×10^{-12}	SYNE1/6q25.1
7	rs74491657	46947633	G	A	0.91	0.78	1.08 (1.03–1.13)	1.23×10^{-3}	1.46 (1.28–1.59)	2.24×10^{-8}	7p12.3
11	rs74485684	30242287	T	C	0.84	0.98	1.11 (1.07–1.15)	2.00×10^{-8}	1.26 (1.15–1.35)	7.77×10^{-7}	FSHB/11p14.1

Chromosome (Chr); single-nucleotide polymorphism (SNP); Genomic position is shown relative to GRCh37 (hg19); genome wide association study (GWAS); risk allele (RA); other allele (OA); odds ratio (OR) with respect to RA; confidence interval (CI); average risk allele frequency in European samples (RAF_{EUR}); average risk allele frequency in Japanese samples (RAF_{JPT},).

2.1.5.2 Epigenomics in endometriosis

Epigenomics focuses on the study of genome wide characterization of changeable alterations of the DNA or DNA associated proteins, as well as the inherited changes in gene function that are not associated with changes in DNA sequence. Protein, including histone, binding alterations of DNA are key regulators of gene transcription and consecutively of cellular fate [446]. These alterations may be influenced equally by genetic as well as environmental factors and may be long lasting [447–449]. All the genomic regions within the DNA packaged together with proteins (*e.g.*, histones) are affected by epigenomic mechanisms which give rise to structures known as chromatin. The most characterized epigenomic alterations are DNA methylation (in cytosine of GC dinucleotides) and histone modification. Epigenetic signatures are often tissue specific [450], and there are many large groups concentrating on creating detailed epigenomic maps in several human tissues (Roadmap Epigenomics (http://www.roadmapepigenomics.org/) and International Human Epigenome Consortium (http://ihec-epigenomes.org/)) [317].

Although the role of epigenetic alterations as a mediator of transgenerational environmental effects remains controversial [451,452], their importance is evident in various biological processes, including developmental, physiological, and pathological ones, as well as disease developmental mechanisms from multiple epigenome wide association studies [453,454]. For instance, the differentially methylated regions of the DNA could be used as indicators of disease status for cardiovascular disease [454], cancer [455], metabolic syndrome [453,456], and countless other pathophysiological states [457]. There is mounting evidence that methylation alterations in specific genes, may contribute to the pathogenesis of endometriosis, while in recent years the wider effect of epigenetics in endometriosis has been expansively studied with the possibility to discover the molecular processes leading to the disease [458,459].

Numerous epigenetic studies in endometriosis have centered primarily on the differentially methylated DNA in single genes or genome wide characterization between normal and endometriotic endometrial stromal cells, but not much on histone modifications [365]. A differential regulation of the expression of hundreds of genes in the endometrial and endometriotic stromal cells were discovered with significant differences in their methylation patterns, including a high proportion of these genes encoding transcription factors involved in the pathogenesis of endometriosis [460]. For example higher levels of methylation was shown all through the promoter and coding region of GATA-binding factor-6 (*GATA6*) in endometriosis stromal cells when compared with the endometrial stromal cells, while exons 2 and 4 of *GATA6* displayed full methylation in the endometrium and less methylation in endometriosis [460]. Steroidogenic factor 1 (SF1) has also been discovered to be heavily methylated and below detection level in endometrial

stromal cells, but with up to 12,000-fold higher expression in endometriosis stromal cells [460,461]. Furthermore, the progesterone receptor B (PGE2-d4 RB, encoded by the *PGR* gene) was discovered to be differentially methylated in the endometrial stroma cell as compared to the endometriosis stromal cells, resulting in the suppression of its expression in endometriosis cells [365,460]. *ER-β* encodes estrogen receptor 2 a vital mediator of estrogen action in endometriosis stromal cells is deregulated because of changed methylation in the ectopic endometrial tissue as compared with the eutopic tissue [460,462]. A 142-fold higher *ER-β* mRNA and protein expression levels was detected in endometriosis stromal cells in comparison to the normal endometrium, due to hypomethylated promoter region in the *ESR2* gene [460,463,464]. It has been proposed that the unusually high concentrations of *ER-β* in the endometrium might play a role as a receptive factor for the development of endometriosis [460,465] given the fact that the eutopic endometrium of women with endometriosis have elevated expression of *ER-β* when compared with the endometrium of healthy women. In a recent study, the abnormal methylation of the *IL-12B* promoter region was shown to contribute to the substantially increased mRNA levels detected in eutopic and ectopic endometrium of patients with ovarian endometriosis, therefore associated in the development of this condition [460,466].

2.1.5.3 Transcriptomics in endometriosis

Transcriptomics, which is also referred to as functional genomics, examines RNA levels of gene expression patterns, quantitatively (how much of each transcript is expressed) and qualitatively (identification of novel splice sites, RNA editing sites, which transcripts are present) [317]. In biology the RNA is viewed as the molecular intermediary between DNA and proteins, which are regarded as the key functional readouts of DNA. All the other RNA function, such as regulatory (*e.g.*, X-inactive specific transcript (Xist) a non-coding RNA (ncRNA) unique to placental mammals in X-chromosomes (ChrX) inactivation) or structural (*e.g.*, ribosomal complexes), have frequently been considered as odd exceptions to the general rule. The introduction of large transcriptomic studies over the last decade has demonstrated that although just about 3% of the genome encodes proteins, up to 80% is transcribed [467]. Thousands of novel isoforms have been identified with RNA sequencing studies, these studies have shown a larger than previously appreciated complexity of the protein-coding transcriptome [468] and significantly contributed to the advancement of the field of non-coding RNA. It is clear that thousands of these long non-coding RNAs recorded in the mammal cells (http://www.gencodegenes.org/) play a significant role in various physiological processes. The dysfunction of long non-coding RNAs has been linked with the development of a variety of diseases, such as diabetes [469,470], myocardial infarction [471], cancer [472], and a host of others [473].

In various studies microarray-based genome wide analysis has been used to identify differential expressed genes in the ectopic endometrium compared with eutopic endometrium or in healthy endometrium. Some of these studies have identified genes with altered expression in the ectopic endometrial tissue when compared to the eutopic endometrium, as well as genes that belong to MAPK, PI3K, and RAS signaling pathways [460,474] and other genes associated with neurocrine, endocrine and immunological functions [381]. Numerous differentially expressed genes have also been identified, to be involved in immune cell recruitment, cellular adhesion, T-cell cytotoxicity, cell signaling and apoptosis [337]. In other studies various dysregulated genes that belong primarily to pathways implicated in the regulation of metabolism as well as the action of glucocorticoids and prostaglandins or complete pathway, individually were discovered [336,404,475].

In transcriptome studies aspiring to understand and answer questions on whether there are dissimilarities between the endometrium of patients with endometriosis and healthy women, several genes have been identified with up- or downregulated, expression levels and the differences in their gene expression were found to correlate with stages of the disease [332,333,338,420]. The evaluation of differentially expressed genes in the endometrium of patients with severe endometriosis demonstrated the dysregulation of various pathways, that have already been associated with endometriosis in earlier studies [474]. Equally, dysfunction of the RAS/RAF/MAPK and PI3 kinase signaling pathway genes was shown in other research [332,334,335], connecting the identified pathways with the pathogenesis of the disease. The differentially expressed genes included genes coding for proteins linked with the cell proliferation, cell adhesion, immune system and inflammatory pathways, as well as the components of signal transduction pathways [378].

With the advent of transcriptomics and Next Generation Sequencing (NGS), RNA sequencing (RNA-Seq) has been implemented in studies of endometriosis. RNA-Seq possesses the capability to sequence as well as quantify millions of RNA fragments and, together with bioinformatics, constitutes a valuable tool for comprehensive transcript reads [460].

2.1.5.4 Proteomics in endometriosis

Proteomics is involved with the study of alterations in all proteins expressed as well as converted from a single genome [476] and all proteins released into the neighboring biological fluid [477]. It is used to quantify the abundance, interactions, and modification of peptides. The substantial number of proteins expressed by a cell, or an organism vary from cell to cell and are dependent on the interaction of many factors. The quantification and evaluation of proteins has been transformed through mass spectrometry (MS) based techniques and, have recently been adapted for high

throughput assessments of thousands of proteins in body fluids or cells [477,478]. Affinity purification techniques, where a single molecule can be isolated using a genetic tag or an antibody, is first used before MS to identify any linked proteins. These Types of affinity techniques, occasionally combined with chemical crosslinking, have been modified to *e.g.*, analyze global connections between proteins and nucleic acids (*e.g.*, ChIP-Seq) [317]. Functions of a significant proportion of proteins are mediated through their post translational alterations such as phosphorylation, ubiquitination, nitrosylation, proteolysis, and glycosylation [479,480]. These alterations play a pivotal role in the control of enzyme activity, intracellular signaling, maintaining overall cell structure and, protein transport and turnover [481]. MS may be used to measure such binding alterations directly by specifying the corresponding change in the protein mass. There are efforts to develop genome-level analyses of such modifications [482], using MS-based methods to examine global proteome quantification and interactions of post translational alterations [483,484].

From this perspective, proteomics is thought to be considerably more complex than genomics, given the fact that the genome of an organism remains largely unchanged [460]. Proteomics study is based on the discovery of differentially expressed protein/peptides in different tissues and/or conditions. These changes in protein/peptide expression can be either a precursor to endometriosis or result of this disease [460,485]. Differentially expressed proteins or peptides have been identified in the blood and urine among patients with endometriosis and controls as well as when comparing ectopic with eutopic endometrium [460,486].

In one comparative study, that focuses on identifying proteins expressed in the serum and eutopic endometrium of endometriosis patients in the different stages of the disease vs. controls, eleven proteins were found to be differentially expressed [353]. Furthermore, when the differentially expressed protein profile of the endometrium of women with and without endometriosis were studied in the secretory phase of the menstrual cycle, a total of 119 proteins were found to be differentially regulated between endometriosis and control tissue [354,460]. These proteins were registered in various cellular functions and pathways, including cell structure, transcriptional regulation, apoptosis, and immunity. In the study by Vehmas *et al.* quantitative comparison of eutopic and ectopic endometrial tissues (ovarian endometrioma) discovered 214 differentially expressed proteins between the tissues, with pathway analysis revealing a prospective role for Transforming growth factor β-1 (TGFβ1) in ovarian endometriosis development [387]. Ametzazurra *et al.* examined the endometrial fluid (EF), presenting a complex composition of proteome with over 800 protein spots. Amongst these differentially expressed proteins in the EF between controls and women with endometriosis, were high expression of cytoskeletal proteins. Moreover, changes were detected in the proteins involved in cell cycle regulation and signal transduction, as well as several enzymes involved in various metabolic

pathways [323]. By using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis Zheng *et al.* discovered three peptide peaks (5988.7; 7185.3, 8929.8 *m*/*z*) that differentiated healthy women from women with endometriosis from serum samples [418]. Fassbender *et al.* studied plasma samples in healthy controls and women with endometriosis and reported 18 peptides/proteins in different levels in endometriosis patients as compared with controls [420]. Furthermore, El-Kasti *et al.* using the same experimental platform identified six peptides from urine samples that were able to differentiate between patients with severe endometriosis at stages III or IV versus patients without endometriosis [421]. Wang *et al.* detected five peptides also from urine samples with considerably higher levels in patients with endometriosis versus controls using MALDI-TOF-MS and Liquid chromatography–mass spectrometry (LC-MS/MS) technology [487]. Twenty-two proteins were detected by Cho *et al.*, in the urine with elevated levels in endometriosis patients with vitamin D-binding protein demonstrating the highest differential expression [417]. In a recent proteomic study of ectopic and eutopic endometrial tissue specimens from patients with endometriosis and controls, proteomic alterations associated with endometriosis and the cycle phase were identified in the eutopic tissue from over 1400 identified proteins [460]. There were elevated levels of muscle related proteins in ectopic compared to eutopic tissue, and high expression of extracellular matrix (ECM) proteins in ectopic tissue. Additionally, CA-125 (cancer antigen 125) a glycoprotein detected in human uterine fluid was discovered to be the best single marker for distinguishing between endometriosis and healthy women [488]. Several other proteins have so far been identified in the eutopic endometrium from healthy women when compared with endometriosis patients but very few have been substantiated for a prospective role in the etiology of endometriosis [489].

2.1.5.5 Metabolomics in endometriosis

Metabolomics focuses on the development of techniques to quantify multiple low molecular mass compounds, such as carbohydrates, fatty acids, amino acids, or additional products of cellular metabolic functions in various biological systems. Originally it was defined as the quantitative measurement of perturbations in the metabolite levels of individual cells or cell types in response to stimuli or indicative of different growth conditions, a disease, or drug administration [460,490]. Quantitative measurements of metabolite levels have made it possible to detect new genetic loci that regulate small molecules, or their comparative ratios, tissues and in plasma [491–494]. Furthermore, combining metabolomics with modeling has been used extensively to study metabolite flux. One of the final downstream products of a gene transcription is the metabolome and it is located remarkably close to the actual

phenotype of the organism. Using metabolic profiles, biomarkers for several diseases have been identified, such as for rheumatoid arthritis (Ra), systemic lupus erythematosus (SLE) [495], cancer [496], cardiovascular disease [497], diabetes [498], schizophrenia [499] as well as for several other diseases.

In endometriosis research analyzing metabolic profiles allows for insights into the metabolic modifications associated with the development of the diseases as well as its progression. Using this framework serum metabolome was analyzed in patients with mild endometriosis, 13 metabolites, were identified with significant difference in the serum levels of patients when compared with healthy controls, including amino acids and small metabolites [431]. In another study Prieto *et al.* detected significantly reduced activity of superoxide enzymes and increased levels of vitamin E in the plasma and follicular fluid of women with moderate to severe endometriosis vs. control infertile ones [460,500]. All metabolite studies so far performed have shown significant changes in metabolite profile of endometriosis patients. Dutta *et al.* endeavored to develop a diagnostic marker from eutopic endometrial tissues of women with endometriosis and not from biofluid [501] as was done in previous metabolomics studies on follicular fluid (surrounding the developing oocyte) [502], urine [322], endometrial fluid [503], serum [431] and plasma [504].

2.1.5.6 Lipidomics in endometriosis

Lipidomics, a subsidiary of metabolomics, is classified as the study of pathways and networks of cellular lipids in biological systems [505,506]. It seeks to explore and model lipids at a global level. Lipids are amphipathic or hydrophobic molecules defined by their unique biological and structural properties within cells, with a wide variety of physiological processes which includes maintaining electrochemical gradients, subcellular partitioning, signaling processes, protein trafficking, energy storage, and membrane anchoring [454,506]. The physiological significance of lipids is demonstrated by the various metabolic diseases towards which lipid aberrations contributes, such as diabetes, atherosclerosis, obesity, stroke, hypertension, as well as Alzheimer's disease. Recent developments in soft-ionization mass spectrometry, in combination with established separation techniques, have allowed for sensitive and rapid detection of various lipid species with minimal sample preparation[506]. This fast growing field complements the enormous progress that has been achieved in genomics and proteomics, all of which alongside metabolomics and lipidomics are integral part of the family of systems biology [507].

There have been several lipidomic studies conducted thus far for endometriosis in an attempt to identify endometriosis using lipids as a biomarker. All the studies carried out have discovered alterations in the lipid metabolisms of endometriosis patients suggesting these altered lipids play a vital role in the development of the

disease. One such study when analyzing lipid metabolism in peritoneal fluid, serum, and endometrial tissue of endometriosis patients discovered significant alterations in the sphingolipid metabolism flux in all tissues and a strong Glucosylceramide (GlcCer) correlation in endometriosis patients, signifying GlcCer, a mitogenic factor, could be a major candidate for the survival ectopic lesions [504]. In a different study, eight differential lipid metabolites were discovered in the serum of patients with ovarian endometrioma versus controls among them were the sphingomyelin SMC (16:1), the hydroxysphingomyelins SMOH (C16:1) and SMOH (C22:2), and five ether-phospholipids (acyl-alkyl-phosphatidylcholines) as well as two saturated 2-acyl-1-alkyl-sn-glycero-3-phosphocholines (plasmanylcholines). The authors established a model using several lipids to differentiate between endometriosis and non-endometriosis patients with a sensitivity and specificity of 90.0% and 84.3% respectively [430]. In another study phosphatidylcholines and sphingolipids of the follicular fluid were more abundant in the group with endometriosis in comparison to the controls [502]. Additional study, discovered deregulated levels of various lipids in ectopic tissue in comparison to the eutopic tissue of control, suggesting that the Sphingosine-1-phosphate (S1P) lipid pathway might be crucial in the grafting and survival of endometriosis lesions [508]. A more recent study conducted to evaluate lipid profiles in the endometrial fluids, a total of 457 metabolites were identified of which 123 were found to be significantly differentially expressed in ovarian endometriosis compared to controls. They also reported reduced levels of sphingolipid monohexosylceramides (CMH), ceramides, saturated diacylglycerols and saturated triacylglycerols in endometriosis patients with over expression of glycerolipids and glycerophospholipids. Furthermore, there were increased levels of acyl carnitines also found in the endometrial fluid samples of patients with ovarian endometrioma [503]. Increased levels of acyl carnitines are linked to cell beta-oxidation dysfunction, with previous lipidomics studies substantiating that the different concentrations of acyl carnitines are due to the effectiveness of the mitochondrial membrane bound enzymes involved in the process of beta-oxidation connected to various levels of inflammation [430].

2.1.5.7 Microbiomics in endometriosis

Microbiomics is a rapidly developing field in which all the microorganisms of any given population are explored together. The human skin, the gut, and mucosal surfaces, are all populated by microorganisms, which includes bacteria, fungi, and viruses, collectively referred to as the microbiota with the genes of these microorganisms forming the microbiome [509]. The human microbiome is immensely complex for instance, the gut contains approximately 100 trillion bacteria from around 1000 different species and identifying the microbiota triggering diseases is a

very complicated endeavor due to the substantial variations between individuals from seed during birth and throughout development, environmental factors, diet, drugs, age etc. [509,510]. Numerous studies have linked disturbances in gut bacteria in a wide range of ailments, including cancer, diabetes, colitis, heart disease, obesity, and autism [511,512].

The human body harbors a host of different microflora that are formed early on in life and play an essential role in the wellbeing of the host [513]. In the last few years there has been substantial interest in microbiomic for endometriosis study in trying to understand how human and microbial genetics interact with each other and the immune system as well as the role microbiome plays in influencing the development of endometriosis. Increasing numbers of studies have suggested that the gut microbiota is not only necessary for physiologic gastrointestinal functions but also as a central regulator of various inflammatory and proliferative conditions [514]. Numerous identifiable microbial flora have been discovered in the reproductive tracts of fertile aged women [515], notably in the vagina, where *Lactobacilli* species are highly dominant in the production of lactic acid that regulates the pH of the vagina [516]. The makeup of the vaginal microbiome was found to be reliant on hormonal changes that are linked to the menstrual cycle [517]. One comprehensive study discovered the microbial flora to be significantly different in the cervical and uterine microbiome of endometriosis patients when compared with healthy controls [518]. In a separate study, 183 distinct bacterial phylotypes were discovered in the microbiome of the endometrium [519]. The imbalance of some these key microbial dysbiosis may be a factor in the inflammatory properties of endometriosis [520].

The gut microbiota may play a substantial part in the pathogenesis of endometriosis beyond its role in regulating the cycling of estrogen [521]. Chadchan *et al.* suggested a significant role for the gut microbiota in actively promoting endometriosis disease development and progression [522]. In another study the associations between endometriosis and significantly reduced levels of the gut microbiota was established and how the reduced levels adds to chronic stress by activating inflammatory pathways in endometriosis patients [523]. Other indications of a possible connection between the gut microbiota and endometriosis was detected in women with high omega-3 polyunsaturated fatty acids (PUFAs) consumption having significantly lower risk for endometriosis [524], a discovery that may be explained in part by the changes induced by diet in the gut microbiome [514]. Identifying specific microbiome profiles associated with endometriosis, may be a valuable tool in the early diagnosis of endometriosis. By understanding the microbiomes associated with the disease and restoring these microbiomes to their healthy state could help reduce inflammation and pain.

2.2 AI based approaches to improve women's health

2.2.1 Artificial intelligence

There is no universally approved designation of artificial intelligence (AI). The word broadly suggests the use of computing technologies to model intelligent behavior that simulates processes associated with cognitive functions or human intelligence, such as reasoning, learning and adaptation, sensory understanding, and interaction with minimal human intervention [525]. As machines become more and more proficient, tasks deemed to require "intelligence" are frequently removed from the definition of AI, a trend commonly referred to as the AI effect [526]. A considerable amount of task performed in the modern era was inspired by Aristotle's attempt to reinforce logic ('right thinking') through his syllogisms (a three-part deductive reasoning) and early studies on the operation of the mind [527]. Programs that allow computers to operate using logic are referred to as artificial intelligent systems. In the 1950s the British mathematician Alan Turing, one of the founding fathers of modern-day computer science and AI termed intelligent behavior in a computer as the ability to realize human-level performance in cognitive tasks, this subsequently became known as the 'Turing test' [528]. John McCarthy coined the phrase "artificial intelligence" (AI) in 1955, describing it such as "the science and engineering of making intelligent machines". He was prominent in the initial development of AI, and along with colleagues established the field of AI in 1956 during a conference on artificial intelligence. The conference delivered what evolved into a new cross-disciplinary research area that offered an intellectual framework for all subsequent computer development and research efforts. In subsequent years computers began to resolve increasingly complicated mathematical problems, then came the slowdown. Later in the 80's, a new golden era restarted, instruments with increasing computational power were developed with immense capability to use of AI in logistic data mining and medical diagnosis [529]. Artificial intelligent techniques such as hybrid intelligent systems, Bayesian networks, artificial neural networks, and fuzzy expert systems, are used in various clinical settings in health care.

AI systems are classified by their ability to imitate cognitive behaviors, the hardware they use, their real-world application, and the concept of mind. Using these features all AI systems can be divided into three categories: Artificial Narrow Intelligence (ANI), Artificial General Intelligence (AGI), and Artificial Super Intelligence (ASI). Early AI research was preliminary focused on AGI and the main representation of AI [530]. However, given the challenges as well as the complexity associated with the development of this kind of AI, many scientists turned their focus

on ANI: the capability of a machine to execute a single task extremely well. Virtually all modern AI health applications are regarded as artificial narrow intelligence [530,531].

AI programming concentrates on three cognitive skills: learning, reasoning, and self-correction. The learning processes concentrates on obtaining data and establishing rules for how to transform the acquired data into useful information. The rules, which are called algorithms, offer step-by-step directives to computers on how to accomplish specific tasks. The reasoning process concentrates on selecting the right algorithm to attain a desired result. The self-correction process is intended to constantly fine tune and enhance the algorithms and guarantee that they deliver the most precise result feasible. The different subsets of AI (**Figure 11**) all use these processes in their applications. The most common subsets of AI include machine learning (ML), deep learning (DL) natural language processing, expert systems, robotics, machine vision, and speech recognition.

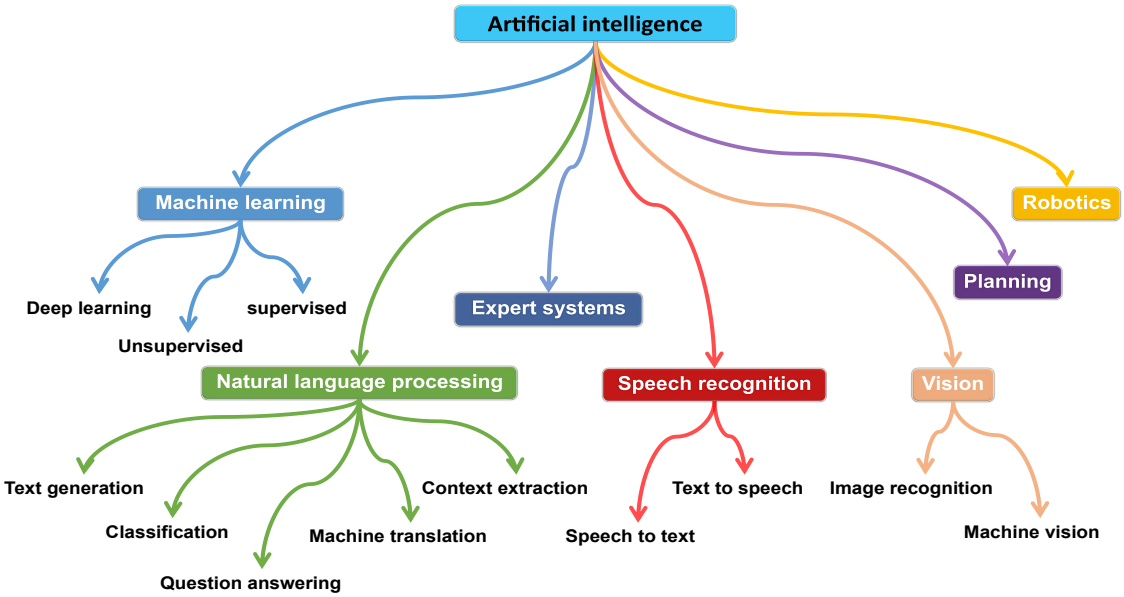


Figure 11. Subsets of Artificial intelligence modified from javapoint.com.

Modern-day medicine is confronted with the challenges on how to acquire, analyze, and apply the enormous amount of knowledge necessary to solving complex problems. The integration of AI into medicine has helped ease some of these challenges by supporting healthcare in everyday tasks, supporting tasks that rely on the manipulation, exploitation, and utilization of data and knowledge [529].

2.2.2 AI in Medicine

As we have become more dependent on computers, it comes as no surprise medicine, like with every aspect of our lives has adopted the computer age with so much enthusiasm and anticipation. The term AI is relevant to a wide range of elements in medicine such as medical diagnosis, robotics, medical statistics, and human biology up to and including today's "omics" [529]. It is creating a model change to healthcare, driven by the growing availability of big data and the fast-paced progress of analytics techniques. It is expected that AI-driven approaches will become much more objective, accurate, and rapid, which will lead to greater precision, standardization, and automatization in medicine. The wider application of AI technology steered by relevant clinical questions, and powerful AI methods could be used to unlock clinically relevant information and complex variables hidden in massive amounts of data, which in turn can help in disease prevention, diagnosing, monitoring patients, and clinical decision making [20,532,533]. Most AI applications are narrow, given that they are only able to accomplish specific tasks or resolve predefined problems. There are 2 major branches of AI in medicine: virtual and physical. The virtual branch includes applications using some subsets of AI such as machine learning, and deep learning, in applications for information management systems, electronic health record, and clinical decision support systems. These systems use mathematical algorithms to improve through learning from experience. Some areas of medicine that use AI tools include cancer, neurology, obstetrics and gynecology, and cardiology.

2.2.2.1 Machine learning

Machine learning (ML) has proven to be the most successful subtype of AI in recent years and is the fundamental approach of many applications, boosting discoveries in all areas of medicine from genetics to molecular medicine. Machine learning algorithms can be further subdivided into three main categories: (I) unsupervised learning (ability to find patterns and or structures in data); (II) supervised learning (classification and prediction algorithms based on knowing the classes or labels from unsupervised learning); and (III) reinforcement learning (use of sequences of rewards and punishments to form a strategy for operation in a specific problem space) **Figure 12**.

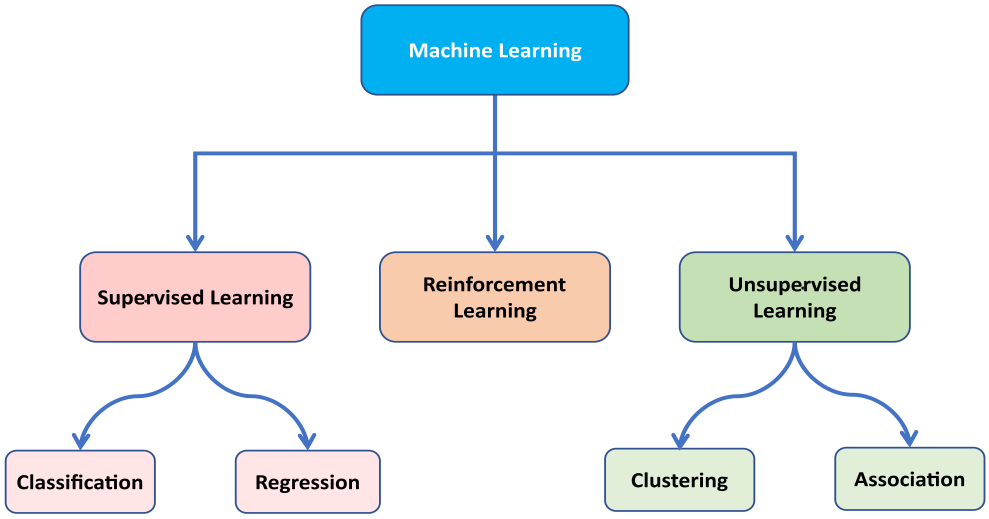


Figure 12. Types of machine learning techniques.

Supervised learning

There are numerous machine learning methods that can be used to learn how to map objects to classes and create predictive models [534]. Supervised learning also known as supervised machine learning employs regression and classification methods to develop machine learning models. to generate reasonable predictions for the responses to new data Models are trained with a known set of input data and known response to the data. As data is fed into the model, the weights are adjusted until the model has been appropriately fitted. This happens within the framework of the cross-validation process to ensure that the model avoids overfitting or underfitting. the classifications or predictions become increasingly more accurate as training progresses.

Classification techniques

Classification algorithms predict direct response, and are applied when output variables are categorical i.e., includes two classes such as yes – no, or whether a tumor is malignant or benign etc. Several of the most commonly used classification techniques include Random Forest (RF), support vector machines (SVMs), boosted and bagged decision trees, naive Bayes classifier, *k*-nearest neighbor logistic regression, discriminant analysis and artificial neural networks, which includes the recently developed deep neural networks [534].

Regression techniques

Regression algorithms predict continuous responses, and they are used when there is a link between the input and the output variables such as, in weather forecasting.

common regression techniques which come under supervised learning include linear regression, non-linear regression, stepwise regression, regularization, adaptive neuro-fuzzy learning, decision tree regression, random forest regression and neural networks.

Unsupervised learning

Unsupervised learning is a technique used to draw conclusions from data without labeled responses i.e., models are trained using unlabeled datasets and then allowed to act on the data without supervision. It is also utilized in dimensionality reduction processes to reduce the number of features in a model. Unsupervised learning algorithms can be divided into two types: clustering and association.

Clustering is the most widely used unsupervised learning technique and frequently employed in exploratory data analysis to find groupings or hidden patterns in data. The association rule on the other hand, is used to find correlations between variables in large datasets by determining the set of items that appears together in the data. k-means clustering and k-medoids, hierarchical clustering, principal component analysis (PCA), singular value decomposition, Gaussian mixture models, hidden Markov models, anomaly detection, fuzzy c-means clustering, subtractive clustering, and more are examples of common unsupervised learning algorithms.

Machine learning process

Training, validation, and testing

To make accurate predictions ML algorithms require quality training and testing data, and to prevent overfitting, datasets are separated into train, validation, and test splits. While all three are typically split from one large dataset, each one has its own unique application in ML modeling. The training dataset is the sample of data used to fit or train the model. This data is seen and learned by the model. The validation dataset is a subset of data that is used to offer an unbiased assessment of model fit on the training dataset while setting model hyperparameters. As skills on the validation dataset are incorporated into the model configuration, the evaluation becomes increasingly biased. The test dataset, on the other hand, is a subset of data used to offer an unbiased assessment of the final model on the training dataset. It is only used once a model is completely trained using both the train and validation sets.

Overfitting

Overfitting is one of two key issues in machine learning that diminishes the performance of ML models. The goal of a ML model is to generalize well and the ability of a ML algorithm to make predictions and classify data is attributable to the generalization of a model to new data.

When ML algorithms are developed, a sample dataset is harnessed to train the model. However, if the model is trained on sample data for an inordinately prolonged period or if the model is overly complicated, it may begin to learn extraneous information or "noise" within the dataset. When this happens the model's ability to generalize well to new data diminishes and performance degrades. The model on the other hand is unable to accomplish the classification or prediction task for which it was designed. Low error rates and high variance are good indicators of overfitting.

While using linear models can help avoid overfitting, many issues where ML are applied to find solutions are nonlinear. Feature selection, cross-validation, training with more data, data augmentation, early stopping, ensemble methods, and regularization are a few methods used to reduce the occurrence of overfitting in ML.

Regularization

Regularization is one of the most fundamental concepts of ML. It is a technique used for preventing ML models from overfitting by adding more information. It can be employed in such a way that it allows all variables or features in a model to be maintained by lowering the magnitude of the variables. Consequently, the accuracy and generalization of the model are maintained. Regularization mostly reduces the coefficient of features to zero. Simply put, the regularization technique minimizes the magnitude of the features by maintaining the same number of features. Regularization techniques are classified into two types: ridge regression and lasso regression.

In ridge regression a modest amount of bias is introduced to improve long-term prediction. Ridge regression is used mostly to reduce overfitting in ML models which contain all features in the model. It decreases the model's complexity by decreasing the coefficients.

Lasso regression is like ridge regression, except that the penalty term only contains absolute weights rather than a square of weights. Lasso regression aids in the reduction of overfitting in both ML models and feature selection. Because lasso regression uses absolute values, it can reduce the slope to zero, whereas ridge regression can only reduce it to near-zero values.

Feature selection

Is the selection of a subset of available features for use in model creation. When presented with many features that are not critical for predicting the desired output, practical ML algorithms are known to degrade in performance (prediction accuracy) [535]. In feature selection, the best subset of features has the fewest dimensions that contribute the greatest to prediction accuracy. Feature selection techniques are used for a variety of reason, including shorter training periods, better data compatibility with a learning model class, avoiding the curse of dimensionality, and more.

The curse of dimensionality, which is one of the reasons for using feature selection, refers to a variety of phenomena that emerge while evaluating and organizing data in high-dimensional domains. Feature selection is different from model evaluation. As a result, it is critical to ensure that predictive models are evaluated on data that has not been used to estimate model parameters (training) [536]. This is often accomplished by withholding a subset of data for testing once or more times (e.g., in cross-validation).

Cross validation

Cross validation (CV) is a technique for validating a model's efficiency by training it on a subset of input data and evaluating it on an unknown subset of input data. Simply said, it is a way for determining how well a model generalizes to an independent dataset. In machine learning, it is always necessary to validate a model's stability. A model is given a dataset of known data on which to train (training dataset) and a dataset of unknown data against which the model is evaluated in a prediction job (validation dataset or testing set). CV assesses the model's ability to predict new data that was not utilized in estimating to identify issues such as selection bias or overfitting. CV is classified into two types: exhaustive and non-exhaustive. Exhaustive CV methods learn and evaluate every viable way to partition the original sample into training and validation set. Exhaustive CV techniques include leave-p-out cross validation and leave-one-out cross validation. Non-exhaustive CV algorithms do not compute every viable way to split the original sample. These methods are close approximations to the leave-p-out CV method. K-fold CV, stratified k-fold CV, and the Holdout method are examples of Non-exhaustive CV.

2.2.3 Machine learning in healthcare

In its application ML has led to discovery of novel therapeutic targets using unsupervised protein–protein interaction algorithms [537]. New methodology is also being developed using ML evolutionary embedded algorithms which are more robust and less susceptible to over-fitting to identify DNA variants as predictors of diseases or traits [529,538]. Deep neural networks have been used in the discovery of genetic variants [539] from large scale genomic data as well as extrapolate the functional impact of germline and somatic genetic variants [534,539–541]. Machine learning methods could soon be used in the future to predict responses to therapy based on genomic characteristics [542,543]. ML has become instrumental in developing precision medicine especially in medical imaging. For instance, AI applied to images of skin lesion can predict whether or not the lesion is malignant [534,544], same goes for AI applied to retinal scans can predict diabetic retinopathies and other retinal diseases with relative high accuracy [545,546]. When applied to pathology data (tissue images), AI can be used to predict whether tumors have certain genetic alterations

[547], diagnose disease from radiology images [534,548,549], differentiate between different cancer subtypes [550], and even identify polyps in colonoscopy videos [551].

The physical branch includes physical objects, medical devices and increasingly sophisticated robots used in performing surgeries, in intelligent prostheses for handicapped people, in drug delivery system using targeted nanorobots, in monitoring effectiveness of treatments, in physical rehabilitation assessments and elderly care [529,552–555]. The overwhelming majority of these novel AI applications in medicine require further research, especially in the areas of human computer interactions.

Aside from simply demonstrating superior effectiveness and efficiency, modern technologies entering the medical field must also integrate with existing practices, obtain relevant regulatory approval, and, perhaps most importantly, inspire health care professionals and patients to participate in a new paradigm. These challenges have resulted in several new developments in AI research and implementation. AI is not a substitute for clinical experience but can assist physicians in decision-making by providing the most up-to-date medical information about clinical practices to proper inform patient care as well as to make more confident decisions. AI system can potentially help decrease diagnostic delays and therapeutic errors that are unavoidable in clinical practice by enhancing the precision in interpretation and reducing the amount of work that could lead to details being overlooked [533,556–562]. Today, the first signs of AI's impact in healthcare are already becoming apparent – and the potential advances available are significantly greater than anything delivered in recent decades. However, only the very surface has been scratched so far [563].

2.2.4 AI application to women's health

In the various fields of medicine, there has been exponential growth in the use of AI, especially with regards to the diagnostic or predictive analysis of medical data [564]. AI use in medicine ranges from online check-ins in medical centers, scheduling of appointments, reminder calls for follow-up appointments, digitization of medical records, to drug dosage algorithms and adverse effect warnings while prescribing multidrug combinations and research.

Implementation of AI in forecasting health outcomes in women's health has been investigated extensively in the areas of in-vitro fertilization (IVF), pregnancy surveillance, preterm labor, parturition, fetal heart monitoring, gestational diabetes mellitus, cancer screening, cardiovascular, breast, cervix, skeletal health, endometrium, and gynecological surgery.

Artificial neural networks (ANNs) a subset of ML has proven to be one of the most widely used AI technique in medicine [565]. ANNs are ML techniques that imitate aspects of how the brain works [566]. They are a network of neurons (interconnected

computer processors) that can perform parallel computations for data processing and knowledge representation inspired by the nervous system. A neural network usually consists of multiple layers (input, hidden, and output layers) of artificial neurons completely linked to each other by edges, with each one associated with a weight. Every neuron receives signals from multiple neurons in the previous layer, integrates those signals, and fires on the condition that the integrated signals are above a specific threshold [534] (**Figure 13**). The ability of ANNs to learn, classify, recognize patterns accurately, handle imprecise information, analyze non-linear data, and generalize independent data has made them an extremely attractive analytic tool and has drawn scientists to implement them in resolving numerous clinical problems. ANNs have been applied to diagnosing cytological and histological samples for example PAPNET, an automated screening system developed based on neural networks to assist cytologist with cervical screening [567,568].

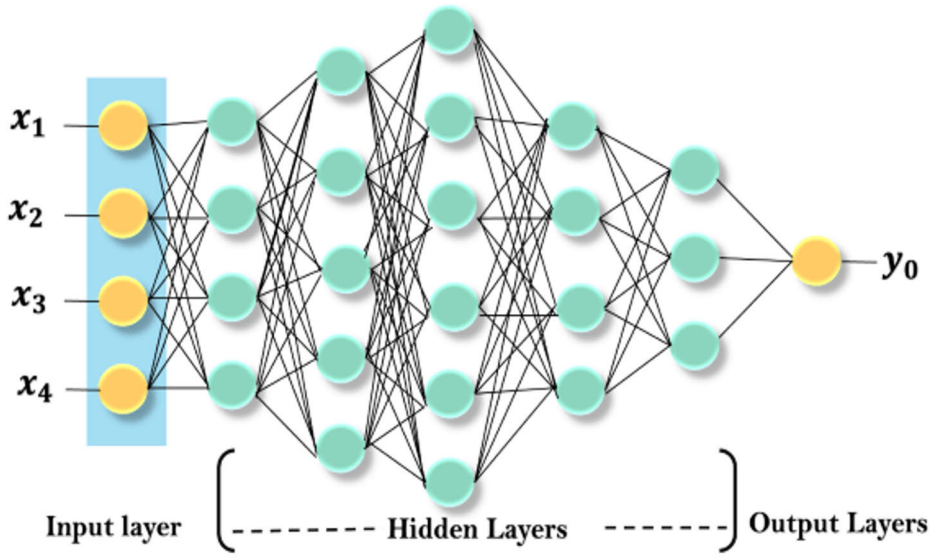


Figure 13. Artificial neural network architecture. Source javapoint.com

ANNs have also been applied to the prediction of endometrial cancer in postmenopausal women and in estimating the effect of human papillomavirus (HPV) types in influencing the recurrence risk of cervical dysplasia [564]. As well as in breast imaging where images from mammograms, sonograms and magnetic resonance imaging (MRI) were used to improve diagnostic performance [564,569]. ANNs have been utilized to predict congenital heart disease risk in pregnant women with the model identifying patients with high risk of developing congenital heart disease early

on in pregnancy [564]. ANNs have been used to interpret plain radiographs [570], ultrasound [571], computer tomography (CT) [572], MRI [573], and radioisotope scans [574].

ANNs pattern recognition has been used in analyzing various wave forms including the interpreting electrocardiograms (ECGs) to diagnose myocardial infarction [575], atrial fibrillation [576], and ventricular arrythmias [577], and to predict a patient's risk after acute coronary syndrome. support vector machines and logistic regression has been used in risk predictor models for stroke, heart failure and renal failure [564]. Analysis of electroencephalograms (EEG) by neural networks has led to its application in the diagnosis of epilepsy [578] and sleep disorders [579]. They have also been instructed to analyze electromyographic (EMG) [580] and Doppler ultrasound [581] wave forms as well as hemodynamic patterns in intensive care patients [582].

From discriminating gastric cells [583], examining thyroid lesions [584], categorizing oral epithelial cells [585], identifying urothelial cells [586], pleural and peritoneal effusion cytology [587] to have all been subject to analysis using neural networks with varying degree of success.

Predictive models for osteoporosis have also been developed to help screen for the risk of osteoporosis or fractures as well as assessment of bone age in evaluation of patients with endocrine and metabolic disorders [564]. Machine learning has been used in preventive medicine to predict which patients were at increased risk colorectal cancer and complications of type II diabetes such as retinopathy, neuropathy, and nephropathy [564]. In reproductive medicine there are ongoing studies on the role and impact of AI in personalizing treatments for infertility with accurate prediction for live birth, embryo implantation potential, effect of endometriosis on outcomes of assisted reproductive technology (ART) [564,588]. In ART deep learning have been applied to predicting the quality of blastocyst based on static [589] or time-lapse embryo images with high accuracy in individual patients [534,590,591]. A convolutional neural network (CNN) can be taught to identify particular areas in the embryo, such as the trophectoderm, and the inner cell mass, which is subsequently fed into an algorithm to assesses the quality of embryo [534,592].

In endometriosis there are multiple studies where AI have been used to predict the risk in women with clinical information as well as pregnancy rates following surgical diagnosis of endometriosis [236–242,252–255,593–595]. However, none have been able to produce a simple analytic approach to aid clinical decision making.

3 Aims

This study is an integral part of our long-term objective to discover novel non-invasive diagnostic and prognostic methods for endometriosis. To achieve this objective, we developed a relational database management system "ENDOMET database" that incorporates data from more than 300 patients with endometriosis and more than 100 controls with over 10 million data points. These data points include clinical features with 162 different parameters, including detailed surgical findings, clinical description of symptoms, fertility history, medication history, hormonal medication status, disease stage etc. from both patients and controls, detailed histopathological data from the eutopic and ectopic endometrium. The data also includes, biomarker data from biofluids and various tissue and lesion types (endometrium, peritoneum and endometriosis lesion), such as genome wide expression data from 190 lesions and from 120 endometrium and 60 peritoneum biopsies from both patients and healthy controls; metabolomic data with analytes measured from serum and peritoneal fluids; proteomics data measured from ovarian endometriomas and peritoneal fluids; serum concentrations for 29 different cytokines and growth factors, from serum and tissue; serum concentrations for various steroid hormones; serum concentrations – for CA-125, HE4 and 6 cancer markers, as well as 5 year post surgery follow up data.
Facilitated by the data collected in the ENDOMET database, we aimed to:

1. Generate an endometriosis research resource tool, a database and provide a web-based graphical user interface with an analysis engine to facilitate the wide use for gene expression and serum biomarker analyses with limited statistical analysis expertise. (I)
2. Identify novel molecular markers for defining lesion properties and lesion growth. (I, II)
3. Develop a machine learning model that combines both clinical features and biomarker data to aid the diagnosis of endometriosis more efficiently. (I, III)

Successful implementation of these goals is expected to lead to an improvement in the diagnosis, treatment, and well-being of endometriosis patients.

4 Materials and Methods

A complete description of the materials and methods used in this dissertation are included in each of the original publications I–III. The original tables and figures are cited with italics in parentheses.

4.1 Study design (I, II, III)

All studies are part of the ENDOMET project ("Novel diagnostic tools for endometriosis and their exploitations for prognosis and prevention of complications"). The ongoing study is conducted at the Department of Obstetrics and Gynecology, Turku University Hospital, University of Turku, Finland, and the Institute of Biomedicine, Research Centre for Integrative Physiology and Pharmacology, University of Turku, Finland. For the studies presented in this thesis samples were collected between 2005 and 2015 in collaboration with Helsinki University Hospital, Turku University Hospital, Pohjois-Karjala and Päijät-Häme Central Hospitals.

Study protocol has been approved by the Joint Ethics Committee of Turku University, and Turku University Central Hospital in Finland and are registered in ClinicalTrials.gov with the trial number NCT01301885. A written informed consent for participation in the study was required from all the study subjects for data collection and storage as well as for fluids and tissue samples prior to surgery. Specimen collection was conducted together with the Auria biobank (https://www.auria.fi/en/index.php). The sample collection protocol looks very comparable to those suggested by the World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonization Project and the Endometriosis Phenome and Biobanking Harmonization Project WERF/EpHECT [216,217,596–598], in spite of carrying out the collection before those recommendations have been published.

4.2 Study subjects (I, II, III)

Participants were aged between 18 and 48, prior to surgery, serum samples from all study subjects were collected into non-heparinized tubes and centrifuged for 15 min at 800 g after being kept for no longer than 30 minutes at room temperature and later stored at -20°C or for long term at -80°C until analyzed. Samples of healthy or normal endometrium, and peritoneum as well as endometriosis lesions were collected from endometriosis patients, at all participating hospitals. From control women, healthy tissues from the endometrium and peritoneum were obtained from women undergoing laparoscopic tubal ligation and from those undergoing surgery for benign ovarian cysts, dermoid cysts, and pelvic pain at the Turku University Hospital, University of Turku, Finland. Endometrial biopsy had been taken after the induction of anesthesia utilizing a sterile endometrial sampler (Pipelle de Cornier®; Laboratoire CCD, Paris, France, www.ccd-international.com) to assess the menstrual cycle phase and to eliminate endometrial pathology. The menstrual cycle phase was classified into four subcategories: proliferative, secretory, menstrual and medication, atrophic, inactive, or insufficient.

A conclusive diagnosis was reached through laparoscopy or laparotomy, and endometriosis was further confirmed through histopathological evaluation of obtained samples in the two cohorts. Endometriosis was excluded through laparoscopic surgery during tubal sterilization in healthy women in cohort I and during surgery for benign ovarian cysts, dermoid cysts, and pelvic pain in cohort II. The stage of the menstrual cycle was determined on the day samples were obtained using a questionnaire, endometrial histology, and serum progesterone concentration. Urine samples and peritoneal fluid samples were also collected from all participants.

Pregnancy, acute pelvic infection, suspected malignancy, and other significant diseases were used as exclusion criteria. Preoperative diagnostic protocol was conducted in accordance with standard clinical practice, with a minimum of a gynecological examination in combination with TVUS for all patients. The severity of the disease was determined using the revised American Society for Reproductive Medicine (rASRM) classification system and classified into stages (Stages I-IV). Additional pathologies, such as benign ovarian tumors or fibroids, were documented during surgery. Women with suspected endometriosis but no observed lesions in laparoscopy were considered as healthy controls. Women with unexpected asymptomatic endometriosis in sterilization were included as patients.

4.3 Clinical examinations and sampling

The timeline of the patient examination and sampling, and the overlap of data used in studies I, II, and III are shown in **Figure 14**. During the out-patient visit if

endometriosis is suspected, the patient signs an informed consent (required for ethical reasons, stored at the hospital), and is given an identification code. This patient identification code is stored in the EndometDB and links the patient to their data. The EndometDB does not contain sensitive patient data like names or social security codes. Before surgery, the patient fills a questionnaire (in Finnish: potilaan esitietolomake) (F1) in Webropol. The data is later imported into EndometDB.

Urine and blood samples are collected from each patient before the operation and tissue samples are taken during the surgical operation, after which the surgeon fills the operational forms 1, 2 and 3 (F2, F3, F4) and the fertility EFI (F5) index form after the operation using Webropol.

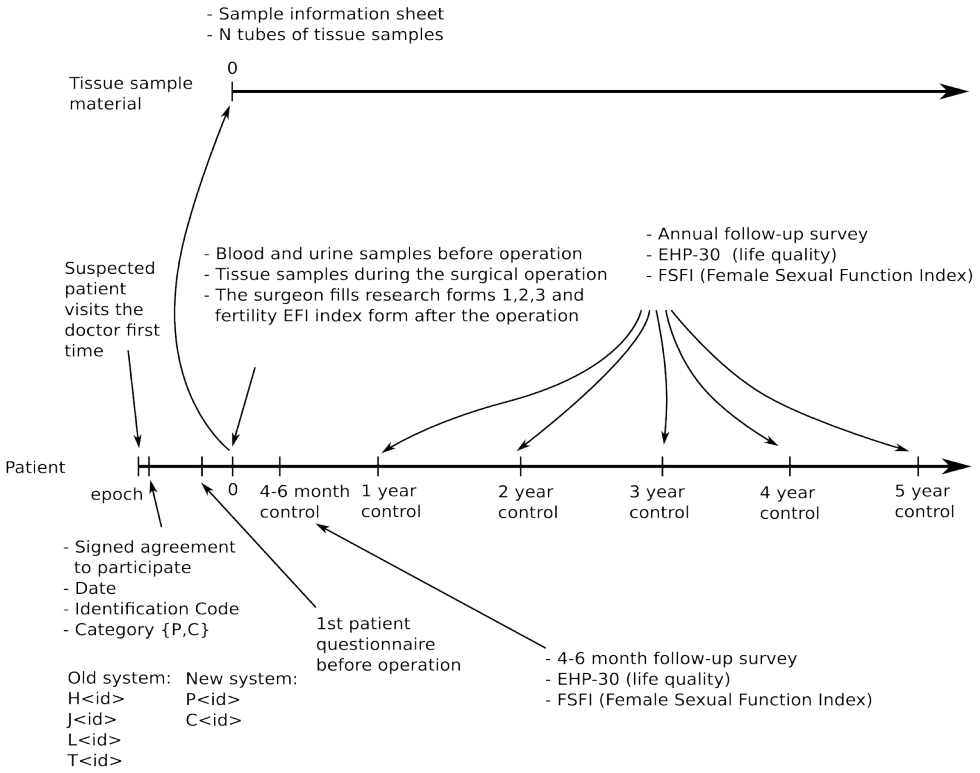


Figure 14. Patient timeline. 4-6-month control survey and annual follow-up survey though collected were not used in the present study.

Subjects are initially classified as [P]atients suspected of endometriosis and [C]ontrols until histological confirmation from a pathologist. This information is submitted to the database using the system user interface (UI).

After 4-6 months post operation a control survey (F6) is submitted by the patient through webropol and annually thereafter the patient submitted a patient survey (F7), quality of life survey (F8), and female sexual function index survey (F9), through Webropol corresponding the following five years.

4.4 Tissue samples

For all three studies, various endometriosis sample subtypes were collected: 1) peritoneal endometriosis lesions, including red peritoneal endometriotic lesion (PeLR), black peritoneal endometriotic lesion (PeLB) and white peritoneal endometriotic lesion (PeLW); 2); deep infiltrating endometriosis lesions (DiE), including deep rectovaginal (REV), sacrouterine ligament lesion (SuL), intestinal endometriotic lesions (DiEIn) and deep endometriotic lesions in the bladder (DiEB) and 3) ovarian endometrioma samples (OMA). Healthy endometrium samples from both patients (PE) and healthy controls (CE) were collected, as well as healthy peritoneum samples from both healthy controls (CP) and patients (PP). All tissue samples were snap-frozen and stored in liquid nitrogen within 10 min, until used.

4.5 Patient characteristics

We used data from 2 cohorts, 277 endometriosis patients and 90 healthy controls prospectively recruited between 2005 and 2015 for the studies presented in this thesis. The women in the control group were not considerably older than those in the patient group with a mean age of 33.5 years and 36 years, respectively (P-value 0.0039). Median range of the body mass index (BMI) between both groups did not differ (p=0.3). The mean height between both groups was 166 cm. Over half of the entire study population were on some form of hormonal medication (56%). Among the groups 27% were on combined oral estrogen and progestin contraceptives, 11% of the women had levonorgestrel-releasing intrauterine device (LNG-IUD) and 12% of the women had been prescribed pills with progestin only. The remaining women on hormonal medications were categorized into combination groups with either gonadotrophin releasing hormone (GnRH) analogues, aromatase inhibitor or a combination of any of the above. Between the different medication groups the stages of endometriosis were not different. Patient characteristics, r-ASRM stages, indication for surgery and menstrual cycle phase presented in **Table 7**.

Table 7. Patient characteristics.

Parameter	Patient group (n=277)	Control group (n=90)
Mean age (SD, range)	33.5 (7.0, 33-34.3) +	36.0 (7.2, 34.4-38) [a]*** ++
Median BMI[b] (range)	24 (24.1-25.2)	24 (24.2-26.4)
Mean height (SD, range)	166 (6.2, 165.2-167) #	166 (6.4, 165-167.3) ##
Parous (%)	99 (27%) ¤	67 (18.3%) ¤¤
Nulliparous (%)	156 (43%)	20 (6%)
r-ASRM stage		
I	50 (13.6%)	NA
II	31 (8.5%)	NA
III	57 (15.5%)	NA
IV	131 (35.7%)	NA
Missing Data	8 (2.2%)	NA
Indication for Surgery		
Pain	184 (50.1%)	NA
Infertility	14 (3.81%)	NA
Both pain and infertility	40 (11%)	NA
Clinical findings	34 (9.26%)	NA
Not recorded	2 (0.54%)	NA
Menstrual cycle phase		
Proliferative	26 (7.1%)	10 (2.7%)
Secretory	37 (10.1%)	16 (4.4%)
Menstrual	7 (1.91%)	1 (0.3%)
Breast feeding	NA	2 (0.54%)
Inactive, atrophic, insufficient, or medication	170 (46.3%)	45 (12.3%)
Missing Data	37 (10.0%)	16 (4.4%)

Note: BMI = Body mass index; NA = not applicable; NS = not significant, [a]*** 0.0039, Two-sample *t*-test, [b] BMI missing 8 in the patient group and 4 in the control group, + 1 (0.3%) missing in patient group, ++ 2 (1%) missing in control group, # 5 missing in patient group, ## 3 missing in control group, ¤ 22 (6%) missing in patient group ¤¤ 3 (1%) missing in the control group.

4.5.1 Study I

In this study a total of 115 endometriosis patients with 53 healthy controls from cohort I were used in microarray analysis. The mean age of the endometriosis

patients was 32 years and 39 years for healthy controls, respectively (p<.0001). The endometriosis patients were classified using the revised American society for reproductive medicine classification (r-ASRM) [10] criteria. In the patient group 15 (8.9%) were in stage I, 15 (8.9%) in stage II, 26 (15.5%) stage III and 56 (32.2%) in stage IV with stages from 3 patients missing. The menstrual cycle phase among both groups were classified into proliferative; secretory; menstrual; and medication, inactive, atrophic, or insufficient. In the patient group there were 19 (11.3%) in the proliferative phase, 26 (15.5%) in the secretory phase, 6 (3.6%) in menstrual phase, and 51 (30.4%) inactive, atrophic, or insufficient. In the control group 14 (8.3%) were in the proliferative phase, 12 (7.1%) were in the secretory phase, 1 (0.6%), and 18 (10.7%) medication, inactive, atrophic, or insufficient.

4.5.2 Study II

In this study 103 endometriosis patient and 47 healthy controls from cohort I were used. Their mean age was 32 yrs. for the patient group and 39 yrs. for the healthy control group, respectively. In the patient group 12 (8%) were in stage I, 15 (10%) in stage II, 25 (17%) in stage III and 49 (33%) in stage IV. 93.6% of the healthy controls were parous with 3% nulliparous and in the patient group 30.1% were parous with 69.9% nulliparous. The cycle phase in the patient group there were 19 (13%) in the proliferative phase, 24 (16%) in the secretory phase, 4 (3%) in menstrual phase, and 46 (30.5%) medication, inactive, atrophic, or insufficient with 4 (3%) in the menstrual phase. In the control group 8 (5.3%) were in the proliferative phase, 12 (8%) were in the secretory phase, and 18 (12%) medication, inactive, atrophic, or insufficient. Forty-four (42.7%) women in the patient group were on hormonal medication and 12 (25.5%) in the healthy control group. Among the patient group there were 33 (75%) on combined hormonal medication, 8 (18.2%) on progestin only, 3 (6.8%) on GnRH agonist and in the healthy control group there were 7 (58%) using combined hormonal medication, and 5 (43%) on progestin only.

4.5.3 Study III

This study was conducted in 2 cohorts with a total of 367 women. In cohort I 139 patients with endometriosis associated pain symptoms: dysmenorrhea (n=128), dyspareunia (n=91), dysuria (n=41), defecation pain (n=73) or abdominal pain (n=74) and 57 healthy controls with a mean age of 32 years and 39 years, respectively. Ten % (19) were in stage I, 9.2% (18) in stage II, 17% (33) stage III and 33.2% (65) in stage IV. Relating to the menstrual cycle phase, in the patient group 10% (19) were in the proliferative phase, 13% (25) in the secretory phase, 3.1% (6) were in menstrual phase, and 32% (62) were put in the medication, inactive,

atrophic, or insufficient category. In the control group 4.1% (8) were in the proliferative phase, 6.1% (12) were in the secretory phase, 0.5% (1) in menstrual phase, and 11% (21) were put in the medication, inactive, atrophic, or insufficient category. Cohort II consists of 136 patients with endometriosis associated symptoms: dysmenorrhea (n=72), dyspareunia (n=109), dysuria (n=36), defecation pain (n=89), abdominal pain (n=79), 2 were asymptomatic women with clinical findings of endometriosis. As healthy controls 33 women scheduled for surgery for benign ovarian cysts, dermoid cysts, and or pelvic pain was used. Mean age of the patients and controls were 32 and 36 years, respectively. Of the patients, 19% (31) were in stage I, 8% (13) in stage II, 14.4% (24) stage III and 40% (66) in stage IV. The menstrual cycle phase in the patient group 4.1% (7) were in the proliferative phase, 7.0% (12) in the secretory phase, 0.6% (1) were in the menstrual phase, and 63.2% (108) were put in the medication, inactive, atrophic, or insufficient category. In the control group 1.2% (2) were in the proliferative phase, 2.3% (4) were in the secretory phase, and 14% (24) were put in the medication, inactive, atrophic, or insufficient category.

4.6 Sex steroid and glycoprotein concentration in serum, peritoneal fluid, and tissue (I)

Serum sex steroid concentrations (estrone (E1), 17-OH-pregnenolone, dehydroepiandrosterone (DHEA), 17-OH-progesterone, androstenedione, testosterone, androstanedione, dihydrotestosterone (DHT), pregnenolone, progesterone, estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), cortisol, and sex hormone-binding globulin (SHBG).) were measured in matched endometrium and endometriosis tissues and in serum samples of the same individuals using liquid chromatography–tandem mass spectrometry (LC–MS/MS) [599]. Twenty mg of frozen tissues were homogenized in 200 µl of sterile water using ultra-turrax (IKA-Werke GmbH & Co. KG, Staufen, Germany), centrifuged (at 3000 revolutions per minute (rpm) for 10 min at 4°C), and extracted with hexane: ethyl acetate (3:2) mixture containing d4-E2, 0.15 ng/ml). The organic phase with lipophilic steroids were evaporated and re-dissolved in 500 µl of 20% methanol in phosphate buffered saline (pH 7.4) for analysis. LC–MS/MS measurements were conducted in Kuopio for review of method see (Häkkinen *et. al* 2018) [600].

4.7 RNA Purification (I, II)

For microarray analysis and quantitative reverse transcription PCR (qRT-PCR), total RNA was isolated from tissue samples using Trizol-reagent (Invitrogen, Carlsbad, CA, USA, Thermo Fisher Scientific, USA), further purified with RNeasy columns

(Qiagen, Netherlands), and treated with DNase (RNase-free DNase Set, Qiagen, Netherlands; or DNase I, Invitrogen, Thermo Fisher Scientific, USA). For the siRNA experiment, TRIsure reagent (Bioline, UK) was used according to manufacturer's instructions. The RNA concentrations were measured using Nanodrop ND-1000 (Thermo Fisher Scientific, USA) spectrophotometer. The quality of the isolated RNA was controlled using Experion™ Automated Electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA), with a mean RNA quality indicator (RQI) value of 7.5 for all the samples.

4.8 Microarray analysis (I, II)

The microarray analysis was performed on 408 tissue samples **Table 8**. For 336 samples the microarray analysis was carried out with Sentrix® Illumina HumanWG-6 v2 Expression BeadChip (Illumina, USA) (I, II) was performed at the Turku Bioscience Center (BTK) and for 72 samples the analysis was carried out using the Illumina HumanHT-12 v4.0 Expression BeadChip (Illumina, USA) microarray platform (I) and was performed at the Biomedicum Functional Genomics Unit (FuGU) of the Helsinki Institute of Life Science and Biocentre Finland at the University of Helsinki. Both the Sentrix® Illumina HumanWG-6 v2 Expression BeadChips (Illumina, USA) and the Illumina HumanHT-12 v4.0 Expression BeadChips (Illumina, USA) microarray platform, contained over 47 000 known genes, gene candidates and splice variants. From each sample three hundred nanograms (*ng)* RNA was used as a template to produce double-stranded cDNA and biotinylated cRNA using the Illumina RNA TotalPrep Amplification Kit (Ambion Inc., Austin, TX, USA). The labeled cRNA were purified and hybridized to the BeadChip at 55°C, for 16 hours following the Illumina Whole-Genome Gene Expression Protocol for BeadStation. Hybridization was detected with Cyanine3-streptavidine (GE Healthcare, Little Chalfont, UK). The hybridized images were scanned using Agilent's microarray scanner and quantified with Feature Extraction Software (Agilent Technology, CA, USA). Raw intensity data was then globally normalized according to manufacturer's instructions. Data from the Sentrix® Illumina HumanWG-6 v2 and Illumina HumanHT-12 v4.0 Expression BeadChips were loaded using *beadarray* R package [601].

Table 8. Samples used in microarray analysis. Modified from Gabriel *et al*. 2020 [602].

Samples	Cycle phase				
Tissue type	**Proliferative**	**Secretory**	**Hormonal medication**	**Others**	**Total**
Control endometrium	14	12	10	7	43
Patient endometrium	16	28	43	14	101
Control peritoneum	3	6	12	3	24
Patient peritoneum	4	9	15	10	38
Ovarian endometriosis	7	9	7	5	28
Peritoneal endometriosis	13	15	37	11	76
Deep endometriosis	9	16	48	13	86

4.8.1 Study I

For global correction, each chip generation was treated as a separate batch. Log transformation and quantile normalization was completed batch-wise using standard R Bioconductor methods [603–605]. We used the BLAST method to map each probe to their corresponding genes utilizing up-to-date gene-to-probe associations. Each probe sequences were aligned to the NCBI's Nucleotide Sequence (*nt*) database [606] implementing a technique published in a previous study [607]. Subsequently aligning to the *nt* database resulted in several hits across multiple species, the data was cleaned and filtered before being used to join the different array generations. To extract the relevant features from the BLAST results, the data was annotated with up-to-date gene symbols and Entrez IDs. Furthermore to achieve a more reliable annotation three different sources are used, dbOrg (bioDBnet - Biological Database Network) [608], HGNC [609] and BioMart (Interface to BioMart databases) [610,611]. During the joining process, the symbols discovered in many of the annotation sources was utilized.

Combining the microarray data from the two Expression BeadChips data frames obtained from the BLAST approach were joined on the Entrez Gene ID and the RefSeq mRNA Accession ID, resulting in 27541 common probes corresponding to 24423 genes. To correct the variation originating in the different Expression BeadChips array versions the ComBat batch adjustment algorithm [612] within the Surrogate Variable Analysis (SVA) R-Package [613] was used. The quality of the merged data was then assessed using PCA and global correlation analysis.

4.8.2 Study II

In study II normalization and analyses of the microarray data were carried out using *limma* R-package [614,615].

4.9 Quantitative real-time polymerase chain reaction (qRT-PCR) (I, II)

For qRT-PCR analysis, 0.5µg of total RNA was converted to cDNA using the DyNAmo HS SYBR Green qRT-PCR kit (Finnzymes, Thermo Fisher Scientific, USA). The qRT-PCR reactions were carried out for 40 cycles at 95°C. For normalization Ribosomal protein L19 (RPL19) and HPRT 1 were used as reference genes. The primers used for qRT-PCR analysis are presented in **Table 9**.

Table 9. Primers used in qRT-PCR analysis.

Primer name	Accession No.	Sense primer (5'→3')	Antisense primer (5'→3')
RPL19	NM_000981.4	aggcacatgggcataggtaa	ccatgagaatccgcttgttt
CYP19A1	NM_001347248.1	agtgcatcggtatgcatgag	agaagggtcaacacgtccac
HSD17B2	NM_002153.3	aactgatggggagcttcttcttat	cctcctcccatgctgctgaca
HSD17B6	NM_003725.4	ctccagcattctgggaagag	aatatgcttgggggcttctt
ESR1	NM_000125.3	tggatttgaccctccatgat	gatctccaccatgccctcta
ESR2	NM_001437.2	tatcacatctgtatgcggaacc	tacatccttcacacgaccagac
AR	NM_000044.4	tggcgggccaggaaagcgac	gggcaaaacatggtccctggca
HGD	NM_000187.4	ctctcaggatcggctttcac	tgtctccagctccacacaag
MPZL2	NM_005797.4	gggacagatgctcggttaaa	caagacacccggtccttaaa
PDGFRL	NM_006207.2	aaaagtggggacgacatcag	gggagattctcgtggtgtgt
SMTN	NM_134270.2	gagtctgcccaagacctcag	agtcttggctcgacaccagt
SRD5A3	NM_024592.5	tccttctttgcccaaacatc	ctgatgctctcccttacgc
TRH	NM_007117.5	ctgaagcgttgtgtgcaaat	agccagacacagcacaacac
STS	NM_000351.5	catggacatatttcctacagtagcc	gatcacgtccatcaatgatcc
PRUNE2	NM_015225.3	cagaaaacatggagctgtgc	aaagggctccagttctaggc
DKK1	NM_012242.4	tccgaggagaaattgaggaa	cctgaggcacagtctgatga
DKK3	NM_015881.5	acagccacagcctggtgta	cctccatgaagctgccaac
FZD7	NM_003507.1	ggctgcgctgcgagaacttc	cagcgcggtgaagggcaggtc
FZD10	NM_007197.3	cctccaagactctgcagtcc	gactgggcagggatctcata
FRZB	NM_001463.4	gcaagcagtgaacgctgtaa	ggcagccagagctggtatag
HPRT1	NM_000194.3	tgctcgagatgtgatgaagg	tcccctgttgactggtcatt
SFRP1	NM_003012.5	cgagtttgcactgaggatga	cagcacaagcttcttcaggtc
SFRP2	NM_003013.3	cgaggaagctccaaaggtat	ctccttcacttttattttcagtgcaa
WNT5A	NM_003392.4	tggctttggccatatttttc	ccgatgtactgcatgtggtc
WISP2	NM_001323370.1	ctgtatcgggaaggggagac	ggaagagacaaggccagaaa

4.10 Primary cell culture and siRNA knockdown (II)

Primary cells from extraovarian samples were isolated from three patients. The samples were plated in serum free DMEM/F12 (Sigma-Aldrich, Merck, USA) supplemented with 1% penicillin/streptomycin and 1% L-glutamine (Gibco, Thermo Fisher Scientific, USA). For primary culture, the samples were rinsed with PBS and carefully minced and digested in 0.25% collagenase solution (Worthington) at 37°C for 1hr. Macroscopically undigested tissue pieces were removed with sterile forceps, and the suspension was then centrifuged, and the cells suspended in PBS. The suspension was then filtered through a 40µm cell strainer to collect the undigested epithelial fraction, which was further digested with trypsin-EDTA for 7 minutes at 37°C, and the reaction then terminated using serum-containing medium. To mimic the whole tissue environment epithelial and stromal fractions were pooled and plated in DMEM/F12 (Sigma) supplemented with 1 % penicillin/streptomycin (Gibco) and 1 % L-glutamine (Gibco, Thermo Fisher Scientific, USA), these cells being of passage (p) 0. When nearly confluent, the cells were split to 24-well plates (p1). siRNA treatments were carried out according to the manufacturer's instructions (Origene, USA) with minor modifications after one passage to 24-well pates. Two different human SFRP2-specific siRNAs were tested using three different concentrations (0.1, 1 and 10 nM). After 24 hours, equal amounts of cells treated with SFRP2-siRNA and control siRNA were seeded into 96-well plates for cell proliferation assay (WST-1; Roche, Switzerland), according to the manufacturer's instructions, and into 24-well plates for RNA harvesting. For Western blot analysis, cells from 2 deep lesions and 1 peritoneal lesion in passage 1 were transferred into a 6-well plate, and within 24 - 48 hours, the siRNAs were added to cultured cells until enough protein could be harvested (11-17 days in culture with siRNAs).

4.11 Western blot analysis (II)

Western blot analysis of 6 ovarian endometriosis samples, endometrium, and extraovarian endometriosis lesions from different patients in both the proliferative (n = 3), and secretory (n = 3) phase of the menstrual cycle were carried out to define the expression of SFRP2. Tissue samples were homogenized on ice with Ultra-Turrax in lysis buffer (150 mM Tris, 150 mM NaCl, 1 % NP-40, 0.5 % sodium deoxycholate, 1 mM EDTA, 1 mM SDS; pH 7.4) containing 1 mM sodium orthovanadate and protease inhibitor cocktail (Complete Mini, Roche), incubated on ice for 30 minutes, and centrifuged at 13000 g for 20 min at 4˚C. Protein concentrations of the supernatants were determined using BCA (Bicinchoninic Acid) kit (Pierce). Thirty micrograms of total protein were separated on a 10-12% SDS-polyacrylamide gel, electrophoresis or 4-20 % precast Mini Protean TGX gels (BioRad) and transferred to PVDF membrane (Hybond-P, Amersham) using a

BioRad semidry transfer apparatus. For cell culture studies, 10–19µg of total lysate per patient were loaded from cells treated with SFRP2-siRNA and control-siRNA. The membranes were treated with rabbit polyclonal antibody against human SFRP2 (0.12µg/ml, HPA002652, Sigma-Aldrich, Merck, USA), rabbit polyclonal antibody against human β-catenin (CTNNB1) (2µg/ml, SC7199, Santa Cruz Biotechnologies, USA) and mouse monoclonal antibody against human tubulin-α (0.02µg/ml, MS-581-P, Thermo Fisher Scientific, USA). After washing with phosphate-buffered saline solution with Tween® 20 (PBS-T), horseradish peroxidase-conjugated secondary antibodies (GE Healthcare and Cell Signaling) were used to bind the primary antibody and protein complexes were visualized with a chemiluminescent detection kit ((ECL, Amersham, GE Healthcare). The intensities of the protein bands were measured using the ImageJ version 1.49.

4.12 Histological analysis (II)

4.12.1 Immunohistochemical analysis

Tissue samples intended for histological analysis, were fixed in 10% formalin, dehydrated, and embedded in paraffin. Five µm thick sections were stained with hematoxylin and eosin (H&E) and utilized in immunohistochemical analysis. Immunohistochemical (IHC) staining was performed using primary antibodies against human SFRP2 (rabbit polyclonal, #HPA002652, Sigma- Aldrich, Merck, USA; 0.3µg/ml for scoring analysis and 0.75µg/ml for lesion border analysis; rabbit polyclonal, #sc-13940, Santa Cruz Biotechnology, USA, 1.3µg/ml to verify lesion border analysis results), human CTNNB1 (mouse monoclonal, #610153, BD Transduction Laboratories, USA, 0.08µg/ml, for scoring and lesion border analysis), and CD10 (also known as MME; mouse monoclonal, #NCL-L-CD10-270, Leica Biosystems, Germany; 0.75µg/ml for lesion border analysis). For IHC the paraffin-embedded sections were deparaffinized and then rehydrated before antigen retrieval. Antigen retrieval was performed in a pressure cooker (Retriever 1200) with Tris-EDTA (pH 9.0) for SFRP2 and CTNNB1, and in 10 mM sodium citrate buffer (pH 6.0) for CD10 for 20 minutes. After cooling for 30 minutes at room temperature, the samples were transferred into hot rinse and allowed to cool for another 5 minutes before a wash in phosphate-buffered saline solution with Tween® 20 (PBS-T, pH 7.4). Blocking against non-specific binding was done using normal goat serum (10% normal goat serum in 3% BSA-PBS-0.05% Tween) at room temperature for 30 minutes. Samples were incubated overnight at 4°C with primary antibody. Endogenous peroxidase activity was inhibited with 1% hydrogen peroxide solution (H_2O_2) at room temperature for 20 minutes. After rinsing in PBS-Tween, sections were incubated with Dako EnVision®+ System-HRP labeled polymer against mouse

IgG, K4001 for β-catenin and CD10 and for SFRP2 Dako EnVision®+ System-HRP labeled polymer against rabbit IgG, K4003 for 30 minutes, washed and stained with Liquid DAB Substrate Chromogen system (Agilent corp., CA, USA) and counterstained with Mayer's hematoxylin. After rinsing with tap water and dH_2O (Distilled water), the sections were dehydrated and mounted. Stained IHC sections were scanned for analyses using the Pannoramic® 250 Flash series digital slide scanner from 3DHISTECH Ltd (Budapest, Hungary). Staining intensity was scored for SFRP2 in endometrium samples with 7 samples in their proliferative and 9 in their secretory phase as well as in the extraovarian endometriosis lesions with 5 in the proliferative and 5 in the secretory phase. For CTNNB1 staining, samples from 3 subjects in the proliferative and 5 in the secretory phase were analyzed from the endometrial and extraovarian endometriosis samples. For the lesion border analysis, extraovarian endometriosis lesion samples from 20 patients were evaluated. Seven were in their proliferative phase, 6 in the secretory phase and with 2 menstruating, as well as 5 samples from women using hormonal medication (combined estrogen and progestin (n = 1), progestin only (n = 2), progestin only + combined estrogen and progestin (n = 1), GnRH analog (n = 1)).

4.12.2 Immunofluorescence

Immunofluorescence double staining for SFRP2 and CTNNB1; and for SFRP2 and CD10 was performed on formalin-fixed paraffin-embedded extraovarian endometriosis samples from 3 patients in the secretory phase using primary antibodies against human SFRP2 (rabbit polyclonal, #sc-13940, Santa Cruz Biotechnology, USA, 1.3µg/ml), human CTNNB1 (mouse monoclonal, #610153, BD Transduction Laboratories, USA, 0.08µg/ml) and CD10 (mouse monoclonal, #NCL-L-CD10-270, Leica Biosystems, Germany, 0.75µg/ml).

Paraffin-embedded sections 5µm thick were deparaffinized and then rehydrated before antigen retrieval. Antigen retrieval was performed using the Retriever 1200 pressure cooker in Tris-EDTA (pH 9.0) for SFRP2 and CTNNB1 double staining, and in 10 mM sodium citrate buffer (pH 6.0) for SFRP2 and CD10 double staining for 20 minutes and allowed to cool for 2 hours at room temperature. Section was then chilled and rinsed with Milli-Q® water (MQ-H_2O) and washed with phosphate-buffered saline solution with Tween® 20 (PBS-T, pH 7.4). After wash, the endogenous peroxidase activity was inhibited by treating the sections with 1% hydrogen peroxide solution (H_2O_2) at room temperature for 60 minutes. Non-specific binding was blocked by incubating the slides with 10% bovine serum albumin in 0.1% PBS-T for 60 minutes at room temperature. Samples were then incubated with primary antibodies in humidity chamber for 60 minutes at room temperature. Sections were then washed in 0.1% PBS-T and then incubated with Dako

EnVision®+ System-HRP labeled polymer against rabbit IgG, K4003 for 60 minutes at room temperature. Samples were then washed in 0.1% PBS-T. Tyramide signal amplification system detection kits (TSA™ Kit #41 with Alexa Fluor® 555 tyramide for SFRP2 and TSA™ Kit #2 with Alexa Fluor® 488 tyramide for CTNNB1 and CD10) were used to stain the samples according to the manufacturer's instructions (Thermo Fisher Scientific, USA) for 10 minutes in humidified chamber and then washed in PBS, before destruction by boiling in MQ-H_2O for 1 minute and then washed in 0.1% PBS-T. All sections were incubated with DAPI at 1:50000 dilution in PBS for 5 minutes, rinsed immediately with PBS and then washed for 5 minutes in MQ-H_2O before being mounted with Invitrogen ProLong™ Diamond Antifade Mountant (P36961, Thermo Fisher Scientific, USA). Analysis was done using the Zeiss Axioimager M1 Epifluorescence and Brightfield Microscope with exposure times set to that of the negative controls without primary antibodies and no signal.

4.13 Serum biomarker and cytokine analysis (III)

CA-125 (Cancer antigen 125 also known as MUC16) and HE4 (human epididymal secretory protein E4; also known as WAP four-disulphide core domain protein 2, WFDC2) concentrations were evaluated using ELISA analysis according to the manufacturer's instructions (Fujirebio Diagnostics Inc, Malvern, PA, USA).

Midkine (MDK) concentration was measured and analyzed in sera from endometriosis patients and healthy controls using a sandwich enzyme immunoassay according to the manufacturer instruction (Biovendor Research and Diagnostic Products, Czech Republic). Elastin microfibril interfacer 1 (EMILIN-1) was measured from serum of endometriosis patients and healthy controls with an in-house sandwich immunoassay (ELISA) using commercially available antibodies listed in **Table 10**.

Table 10. Antibodies used in sandwich immunoassay.

Protein	Category	Clone	Manufacturer	Product number
EMILIN-1	IgG2b κ	5E2	Abnova	H00011117-M03
EMILIN-1	IgG1	60047	Acris	60047-1-Ig

Serum concentrations of 29 cytokines were measured using the commercially available Human Cytokine/ Chemokine Pre-mixed LINCOplex Kit according to the manufacturer's instructions (HCYTO-60K-PMX29; LINCO Research Inc., St. Charles, Missouri, USA). This multiplex assay kit allows for the simultaneous quantitative determination of the following proteins: Epidermal growth factor (EGF), eotaxin, fractalkine, granulocyte colony-stimulating factor (G-CSF),

granulocyte–macrophage colony-stimulating factor (GM-CSF), interferon-gamma (IFN-γ), interleukin-1alpha (IL-1α), IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IFN-γ-induced protein-10 (IP-10), monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1-alpha (MIP-1α), macrophage inflammatory protein 1-beta (MIP-1β), soluble CD40 ligand (CD40L), transforming growth factor alpha (TGF-α), tumor necrosis factor alpha (TNF-α) and vascular endothelial growth factor (VEGF). According to the manufacturer, the intra-assay variation is between 1.6% and 14.6%, and the inter-assay variation is from 5.0% to 15.6%, depending on the analyte.

4.14 Metabolites (III)

Lipid serum metabolites which include (PC.ae.C38.0 and PC.ae.C38.1) being phosphatidylcholines were analyzed from serum of endometriosis patients and healthy controls using targeted electrospray ionization flow injection analysis tandem mass spectrometry (ESI-FIA-MS/MS) with AbsoluteIDQ p150 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria).

4.15 Statistical analysis

4.15.1 Study I

Principle component analysis (PCA) was applied to the microarray data to assess the similarities of each individual chip. Unnormalized and normalized microarray data from each BeadChip as well as on normalized combined data sets were assessed.

4.15.2 Study II

4.15.2.1 Pathway and correlation analysis

In the clustering analysis of the WNT signaling pathway molecules, listed human Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway genes were matched with recently identified WNT signaling pathway genes [616–618]. The CTNNB1 target genes were selected based on the lists of human genes on the WNT home page [619] and in the recent literature [620]. For hierarchical clustering analysis the Ward clustering algorithm and the Canberra distance metric was used. The microarray expression of WNT genes and CTNNB1 target genes were compared among the different tissues using non-parametric unpaired Mann–Whitney test. Multiple comparison-adjusted P-value < 0.05 and thresholds $r \geq 0.3$ were considered as

significant for correlation analyses. Point-biserial correlation was calculated for dichotomous clinical variables such as disease status and fertility. With polyserial correlation calculated for categorical variables such as menstruation length and the number of days with dysmenorrhea. And Pearson correlation calculated for numerical variables such as pain strength, height, and weight. Pearson correlation was also used to investigate the association between intratissue steroid concentrations (estradiol, testosterone, and progesterone) and WNT gene expression using the rcorr function in R software. The SFRP2 promoter transcription factor binding site prediction analysis was done with the ALGGEN PROMO open source software using 0% maximal dissimilarity rate [621].

4.15.2.2 Statistical analysis for data distribution

GraphPad Prism version 7 (GraphPad Software inc., San Diego, CA, USA) was used to perform the statistical analysis. Data distribution was tested using D'Agostino-Pearson and Shapiro Wilk tests, parametric test was selected for normal distribution and nonparametric test was selected for non-normal distribution. Student's t-test or Mann Whitney test was used for two-group analysis and one-way ANOVA or Kruskal-Wallis's test with the appropriate post hoc test (Dunn's multiple comparison tests) was used for multiple group comparisons. Repeated Measure ANOVA (RM-one-way ANOVA) or Friedman multiple comparison test was used to analyze siRNA knockdown effects on SFRP2 mRNA expression and cell proliferation between the different siRNA treatments. Paired t-test was used to compare epithelial and stromal SFRP2 IHC staining intensity during proliferative and secretory phases in endometrium and endometriosis and to compare SFRP2 protein knockdown effect between SFRP2 and control siRNA. Spearman's method was used to analyze correlation between cell proliferation and SFRP2 mRNA expression. The P-value cutoffs for statistical significance were: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

4.15.3 Study III

Statistical analyses for Serum CA-125, HE4, cytokine, MDK, EMILIN-1 and metabolite concentrations were performed using JMP® Pro, version 15.1.0 (SAS Institute Inc., Cary, NC, USA). Data distribution was evaluated using Shapiro-Wilk and Anderson-Darling tests, for normal distribution, parametric test was selected, and for non-normal distribution nonparametric test was used. For multiple group comparisons we used one-way ANOVA or Kruskal-Wallis's test with the appropriate post hoc test (Dunn's multiple comparison tests). Rank analysis of covariance adjusted for age was used to compare each marker concentration between patients and controls, nonparametric tests was chosen, as the values for most markers

were not normally distributed. The concentrations are expressed as medians with interquartile range.

Statistical analyses performed for mathematical model generation were carried out using R language statistical computing environment version 3.4.1 [622].

4.16 PostgreSQL relational database (I, III)

PostgreSQL (https://www.postgresql.org/), an open-source object-relational database management system (ORDBMS) which allows for the handling of various workloads ranging from small-machine application to large internet scale applications with concurrent users. The PostgreSQL database stores information and metadata on a Linux server that efficiently and securely deals with computational demands. An application programming interface (API) was implemented in I and III to allow smooth communication between the server and the clients (web browser on computers, tablets, etc.) specifically adapted to send structured query language (SQL) queries to the database and return the results in standardized format to the client. In addition, the PostgreSQL interfaces with the analysis engine in I which sends custom queries to the database to retrieve measurement data for statistical analysis and visualization.

4.17 Graphical user interface (GUI)

4.17.1 Study I

In I the GUI is implemented on an Ubuntu Linux system that utilizes HTML5, JavaScript, PHP, and R as the main programming languages. The GUI also uses jQuery, Plotly.js, and cascading style sheets (CSS) for frontend styling, and the graph visualizations are generated with the Plotly R open-source graphing library. The GUI allows users, through a client, send requests to the analysis and visualization engine via an application programming interfaced (API) layer implemented with PHP. The analysis engine is implemented as a S3 R package and utilizes several R packages for statistics and graphical outputs, specifically ggplot2 [623]. Plotly and HTML widgets, were used to generate a JSON representation of the plots which is then transferred via Plotly JavaScript Open-Source Graphing Library back to the GUI where it is displayed. The open-source Report Creator App R Package (ORCA) on the backend is used to allow the rendered plot to be generated in PDF. List of programming language and URL in **Table 11**.

4.17.1.1 Interactive visualization

The interactive visualization is implemented using the Plotly open-source JavaScript graphing library (https://plot.ly/javascript/).

4.17.2 Study III

The GUI in III was developed as a progressive web application (PWA). The PWA is implemented on an Ubuntu Linux system and incorporates a GUI built using common web technologies that includes HTML5, JavaScript, PHP, and R as the main programing languages. The GUI also uses jQuery, and Cascading Style Sheets (CSS) for the frontend styling. It requires no installation and works on any platform and can be accessed through any web browser. List of programming language and URL in **Table 11**.

Table 11. List of programming languages used in I, III.

	Programming language		**URL**	**Study**
MAIN PROGRAMMING LANGUAGE	HTML5		https://www.w3.org/html/, https://whatwg.org/	I, III
	JavaScript		https://developer.mozilla.org/en-US/docs/Web/JavaScript	
	PHP		https://php.net/	
	R		https://www.r-project.org/	
	Python		https://www.python.org/	III
FRONTEND STYLING	jQuery		https://jquery.com/	I, III
	Plotly.js		https://plot.ly/javascript/	I
	CSS		https://developer.mozilla.org/en-US/docs/Web/CSS	I, III
GRAPH VISUALIZATION	Plotly R graphing library		https://plot.ly/r/	I
	SVG		https://www.w3.org/Graphics/SVG	III
API	PHP		https://php.net/	I, III
BACKEND	R		https://www.r-project.org/	I, III
	Analysis engine	Plotly graphing library	https://plot.ly/r/	I
		ggplot2 R package	https://www.rdocumentation.org/packages/ggplot2/versions/3.1.1	
		ORCA	https://www.rdocumentation.org/packages/plotly/versions/4.9.0/topics/orca	

4.18 Model generation & Development (III)

All mathematical modeling were carried out using R statistical computing environment version 3.4.1 [622] and Python, version 3.6 [624]. We used predictive models in a binary classification setting to predict the probability of the disease when symptoms are combined with biomarkers. We then used wrapper feature subset selection methodology [625] in particular recursive feature elimination [626] to select the best discriminating clinical features and biomarkers. We tested the model with the wrapper subset selection strategy in a repeated cross-validation setting and ensemble the results across different repeats of the cross-validation. We used nested five-fold cross-validation to train our machine learning classifier for the final classification with RF as the machine learning classifier [627] using scikit-learn in Python [628]. Special attention was taken in encoding variables for clinical data because most of the variables were categorical. Finally, we ensembled the results from the repeated cross-validations into a final list of features to be used for classification.

4.18.1 Data Preprocessing

To produce quality data for the predictive modelling, data pre-processing was applied on each dataset to remove irrelevant and redundant information.

4.18.2 Data Quality Assessment

1. Missing values

   Missing data in the datasets were managed carefully because they can have drastic effects on the final diagnosis. Many of the clinical attributes contained overlapping data which could be deduced using logic and were used to fill in missing attributes. We used the mean value of the attribute to fill in the missing values belonging to the same class for continuous data and for categorical data we replaced the mode of the attribute belonging to the same class. If over 50 % of data were missing for a particular attribute, then it was automatically discarded.

2. Noisy data

   To address noise in our dataset, we used binning. The binning technique divides the dataset in ascending order first and then partitions it into smaller segments (bins) of the same size (frequency) before being applied separately on each segment [629]. Binning, groups multiple continuous values into a smaller number of categorical variables thus reducing complexity and the noise.

3. Duplicate values

   The duplicate values were removed from dataset as the redundant data can result in anomalies.

4. Encoding

   In encoding variables, we performed transformation on our dataset such that it can be utilized as input for machine learning algorithms while retaining its original meaning.

   Categorical variables were divided into 2 types- ordinal and nominal with both encoded differently. To encode ordinal categorical variables, we used label encoder and to encode nominal categorical variables we used One-Hot encoding.

4.18.3 Feature Selection

Feature selection was formulated as: Let $X = \{x_{(1)}, x_{(2)}, \ldots, x_{(D)}\}$ be a set of predictors (features, i.e., clinical features/biological measurements) and T be the target variable (i.e., the disease status we want to predict). The task of feature selection is to find a minimal subset $X'=\{x_{(1)}, x_{(2)}, \ldots, x_{(d)}\}$ of X such that it achieves maximum classification performance of T (for a given classifier and a given classification metric) and $d<<D$.

We used supervised wrapper method to select a subset of the input features in the dataset that are the best predictor for the variables we want to predict training the model and ignoring the irrelevant or redundant ones. With nested cross-validation used to access the predictive contribution of every combination of variables to the result we want to predict.

4.18.4 Classifier Training

Random Forest

A Random Forest consists of an outfit of multiple decision trees that are useful for classification, regression, and also imputing missing value [627]. In our application to endometriosis diagnosis, we used RF in the classification setting where it models a binary response (Patient or Control) as a function of predictor variables (biomarkers and clinical information of patients). In general, decision tree algorithms create a binary tree by continually splitting the features in the dataset into two groups. At every splitting point (a node in the tree), any feature can be split into two groups (children) which results in the best separation between the categorical response. The

splitting is performed until the leaves of the tree contains observations of a single class. Random Forest consist of training a finite number of such decision trees and then finally assembling them to obtain a robust predictive solution.

Decision trees are excellent modelling tools used in classification and prediction and have found usage in many application domains. Decision tree possesses two significant features in biomedical applications, first, it provides a tool for data exploration to identify relationships within limited data and second, decision rules that are generated can be expressed in natural language [630]. Nevertheless, one of the major disadvantages of decision trees is that they are prone to overfitting. Random Forest aims to address this issue by using "Bootstrap aggregating or Bagging" [631] a collection of decision trees, each trained on the different random permutations of the data as well as the predictive features [632]. Finally, the results from these different decision trees are averaged to get the final unbiased prediction, thus improving the disadvantages of a single tree, and providing robust and better generalization capabilities.

4.18.5 Model cross-validation

When the most discerning features have been selected using feature selection, RF was used as the final predictive classifier [627] using scikit-learn in Python [628]. To train the RF classifier, we used nested five-fold cross-validation (CV). Nested CV is frequently used to train a model where hyperparameters also need to be optimized to avoid selection bias [633]. The nested CV projects the generalization error of the underlying model and its (hyper)parameter search. Some RF parameters applied were optimized in the CV setting (*e.g.*, max depth of the trees generated, max features to check for tree splits and the sample count used for bootstrapping), while others were default parameters of the RF method. The data was initial divided into five training and validation sets [633]. Each of the training data was again split into five different folds to tune the parameters of the model in a fivefold CV setting. The model which produced the best results on the test data in the inside validation setup was then again assessed on the validation set in the outer CV setup. The reported outcomes were then averaged over all the repeats of the CV. In the final classification, we used 100 repeats of CV. Each repeat was arbitrarily seeded at each iteration in both feature selection and RF classification for variation in different random parameters (*e.g.*, division of data into test and training set in cross-validation). The reported results are the average of different repeats.

5 Results

5.1 Endomet database (I, II, III)

The Endomet database has one of the most extensive collections of endometriosis clinical and sample data ever collected in a repository for research purpose. It consists of data from over 400 endometriosis patients and healthy controls. With very detailed surgical and clinical descriptions such as menstrual history, initiation of pain, dyspareunia, infertility, medications, previous treatments, and family history as well as continuous annual follow-up of symptoms from all patients. A total, of 2,750 different biological samples of endometrium, peritoneum, endometriosis lesions, blood and/ or serum, peritoneal fluid and urine with extensive clinical descriptions have been collected and entered in the database. Histological evaluation of the endometriosis and endometrial samples and the laboratory documentation for performed analysis are also included in database **Figure 15**.

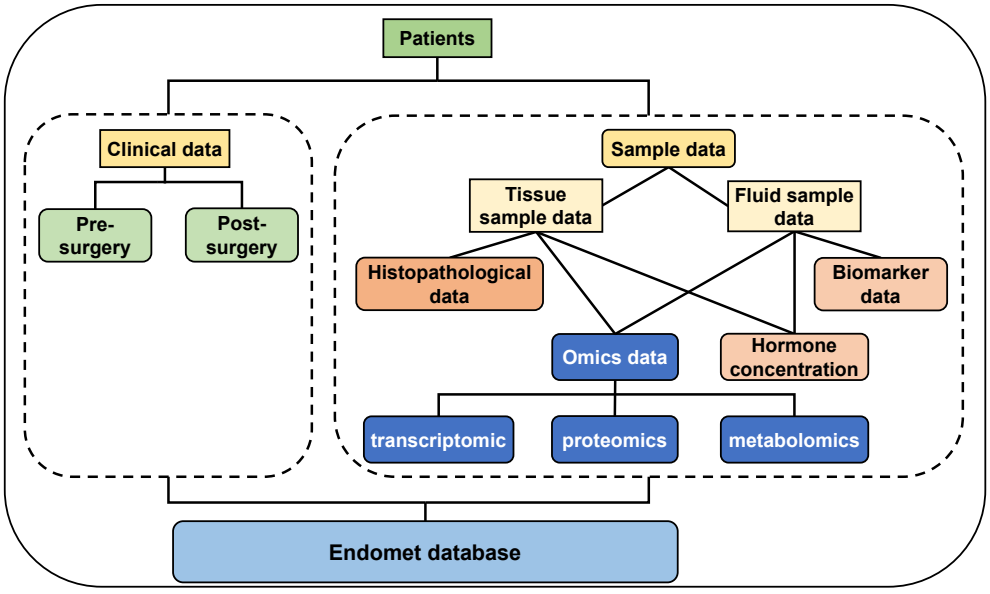


Figure 15. Endomet database structure.

5.1.1 Endomet database system overview

The EndometDB contains and links several kinds of data originating from the endometriosis research. The data accumulates through questionnaires from both doctors and patients as well as measurement data on biological samples that are analyzed using various technologies. All the functionality of the system is available through a Web UI, and access to different functions is restricted using user roles.
The Endomet database dataset includes:

- The whole genome expression profiles performed for eutopic endometrium, endometriosis lesions, and normal peritoneum.
- Hormone concentrations in serum (15 hormones), tissue (13 hormones) and peritoneal fluid (13 hormones) samples.
- Serum cytokine and growth factor concentrations (29 parameters).
- Serum and peritoneal fluid metabolomics (163 metabolites).
- Peritoneal fluid and tissue proteomics.
- Serum concentrations of biomarkers CA125 and HE4.
- Histopathological data for tissue specimens.
- Clinical data: 162 parameters, including detailed surgical and clinical descriptions *e.g.,* pain symptoms, fertility history, medication, hormonal status, and disease stage etc.
- Follow-up of symptoms using annual questionnaires.

The data is stored in the Endomet database through a service layer (API). Analytic functions are available through an analysis engine and can be used to query the whole dataset. The service layer (API) is designed also to be used to integrate the system with new user interfaces (*e.g.*, native mobile app) or other external systems. The data generation and input process of the system is described in **Figure 16**.

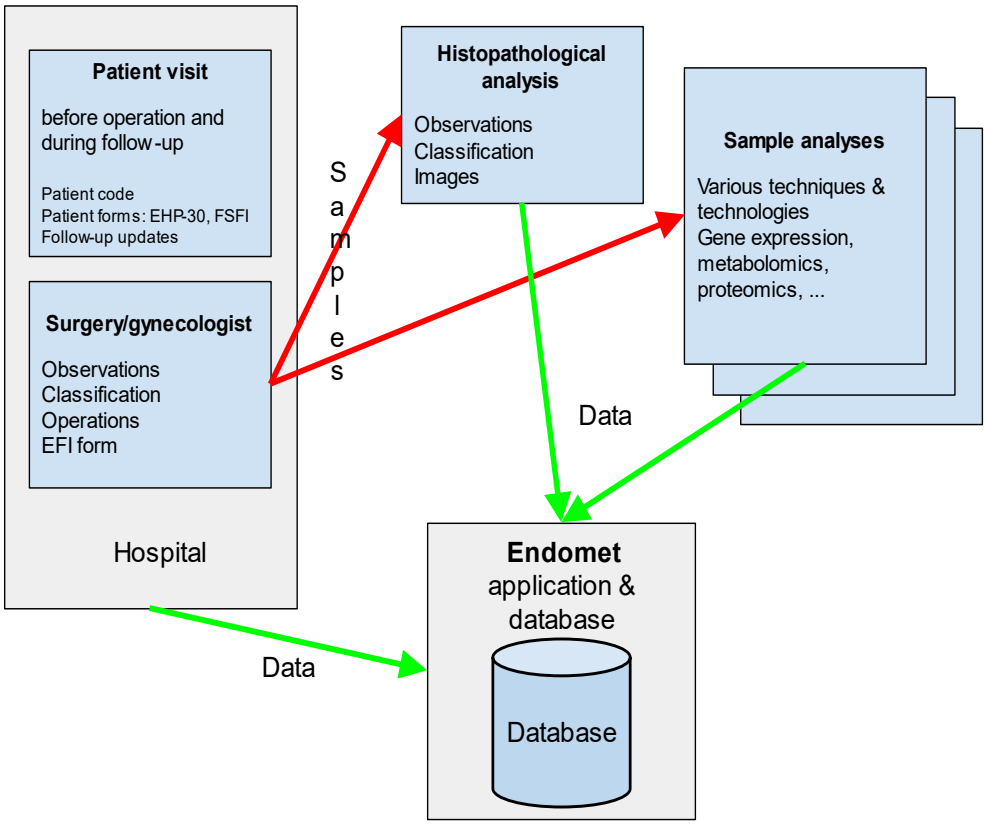


Figure 16. Process overview. The red arrow denotes sample flow in the overall process while the green arrow denotes how data flows.

5.1.2 Endomet database omics

Endomet database omics data includes transcriptomics, metabolomics, and proteomics data from fluid samples (serum, and peritoneal fluid) and tissue samples. The publicly available transcriptomic data contains over 480000 transcripts measured using Sentrix® Illumina HumanWG-6 v2 Expression BeadChip (Illumina, USA), and Illumina HumanHT-12 v4.0 Expression BeadChip (Illumina, USA) microarrays from eutopic endometrium of women with (n=115) and without endometriosis (n= 53), as well as from endometriosis lesions (superficial peritoneal, deep infiltrating and ovarian endometriotic cysts), and macroscopically normal peritoneum of women with and without endometriosis resulting in over 24000 whole genome expression profiles (a total of 408 specimens).

The other omics data in the Endomet database, yet to be made public but used in Study III, contains metabolomics data in the Endomet database contains targeted metabolomic analyses of 163 metabolites in 189 serum and 118 peritoneal fluid

samples from analyzed from 141 endometriosis patients and 66 healthy controls (clinical characteristics in **Table 12**.) measured using targeted electrospray ionization flow injection analysis tandem mass spectrometry (ESI-FIA-MS/MS). The screened metabolites consist of 41 acylcarnitines, 14 amino acids, hexose, 38 diacyl phosphatidylcholines, 40 acyl-alkyl phosphatidylcholines, 15 acyl lysophosphatidylcholines and 15 sphingomyelins.

Table 12. Clinical characteristics of samples used in metabolomics.

Characteristics	Patients (n=141)	Healthy control (n=66)
Age (y), mean, (SD)	32.14 (7.05)	39.15 (4.27) [a] ***
BMI[b] (kg/m^2), median (SD)	22.57 (4.16)	23.18 (4.72)
Disease stage		
Stage I (%) (Minimal)	16 (7.73)	NA
Stage II (%) (Mild)	14 (6.76)	NA
Stage III (%) (Moderate)	32 (15.46)	NA
Stage IV (%) (Severe)	59 (28.50)	NA
Missing (%)	20 (9.66)	
Cycle phase		
Secretory (%)	28 (13.53)	12 (5.8)
Proliferative (%)	22 (10.63)	23 (11.11)
Menstruation (%)	5 (2.42)	1 (0.48)
Medication (%)	54 (26.09)	21 (10.14)
Missing (%)	32 (15.46)	9 (4.35)

Note: BMI = Body mass index; NA = not applicable; NS = not significant, [a] <.0001, *** Two-sample *t*-test, age missing 16 in the patient group and 12 in the control group, [b] BMI missing 3 in the patient group and 3 in the control group.

Proteomics data linked to the Endomet database contains proteins and peptides identified and defined from peritoneal fluid and tissue samples from 13 women with and 4 women without endometriosis in two menstrual phases. The identification of trypsin digested proteins and endogenous peptides from tissues was carried out using nanoflow capillary LC/ESI-MS/MS.

5.1.3 Sex steroid and glycoprotein concentration in serum, tissue, and peritoneal fluid

The Endomet database contains sex steroid concentration measured in the sera, peritoneal fluid, and tissues from endometriosis patients and healthy controls. In the sera 14 hormones (17-OH-pregnenolone, 17-OH-progesterone, androstanedione, androstenedione, cortisol, dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT), estradiol (E1), estrone (E2), follicle stimulating hormone (FSH), luteinizing hormone (LH), pregnenolone, progesterone (P4), and testosterone (T)) and 1 glycoprotein (sex hormone-binding globulin (SHBG)), were measured from 148 endometriosis patients and 76 healthy control women. In the peritoneal fluid 13 hormones (17-OH-pregnenolone, 17-OH-progesterone, androstanedione, androstenedione, DHEA, DHT, estradiol, estrone, FSH, LH, pregnenolone, progesterone, and testosterone) were measured from 60 women with and 14 women without endometriosis and in tissues hormone concentration were measured in the endometrium, and different endometriosis lesion types from 60 women with endometriosis and endometrium from 16 healthy controls.

5.1.4 Serum protein biomarkers, including cytokines and growth factors measured by multiplex assay

Serum concentrations was measured from 124 patients with endometriosis and 53 healthy control women from the following proteins: EGF, eotaxin, fractalkine, granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), interferon-gamma (IFN-γ), interleukin-1alpha (IL-1α), IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IFN-γ-induced protein-10 (IP-10), monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1-alpha (MIP-1α), macrophage inflammatory protein 1-beta (MIP-1β), soluble CD40 ligand (CD40L), transforming growth factor alpha (TGF-α), TNF-α and VEGF.

5.1.5 Serum concentrations of biomarkers measured by ELISA assays

CA-125 (Cancer antigen 125 also known as MUC16) and HE4 (human epididymal secretory protein E4; also known as WAP four-disulphide core domain protein 2, WFDC2) concentrations were measured and evaluated from 125 endometriosis patient and 53 controls. Midkine (MDK) concentration was measured and analyzed from 124 endometriosis patients and 46 healthy controls. Elastin microfibril interfacer 1 (EMILIN-1) was measured from 112 endometriosis patients and 45 healthy controls.

5.1.6 Histopathological data

The EndometDB consists of over 420 endometrium and over 630 endometriosis sample types with histological analysis confirmed by a clinical pathologist. The histological analysis grades the endometrium to determine the menstrual cycle phase.

5.2 Endomet database graphical user interface (I)

The EndometDB web graphical user interface design enables users search and analyze transcriptomic data without the use for advance computational skills. It incorporates different analytical methodologies and techniques, allowing the user to browse and analyze gene(s) of interest in tabbed sections. The EndometDB GUI relies on filter-based data mining that allows mRNA expression of gene(s) of interest in the normal endometrium, and peritoneum from both healthy controls and patients and in various endometriosis lesion types be displayed in several analysis plots using clinical features such as age, menstrual cycle phase, hormonal medication, and disease stage for stratification. These analysis plots include data distribution plots such as boxplots (***I**, Fig. 2*; **Figure 17**) which can be used to show the range of the data distribution and heatmap (***I**, Fig. 3*; **Figure 18**) which can be used to simultaneously compare expression patterns between different pathway genes or genes of interest. These comparisons in the heatmap can be summarized either by the mean or median and centered using the gene or the tissues/lesions. The heatmap can be further analyzed using different unsupervised hierarchical clustering algorithms such as complete linkage; single linkage; average linkage; or ward's method with predefined distance methods such as Euclidean; Canberra; Manhattan; Maximum; and Minkowski method. Clinical features can be used as contrasts in the hierarchical clustering to show how these features relate to the genes of interest.

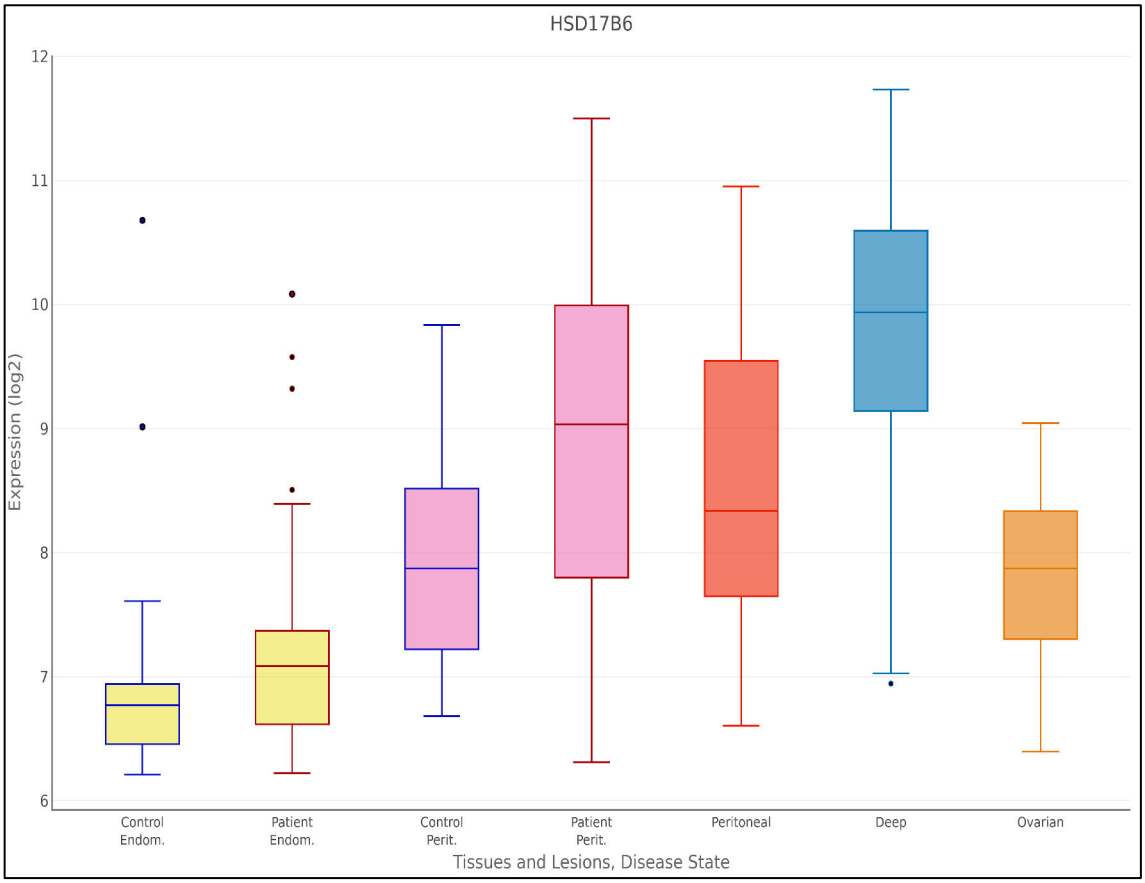


Figure 17. Example boxplot output of HSD17B6 from the EndometDB GUI.

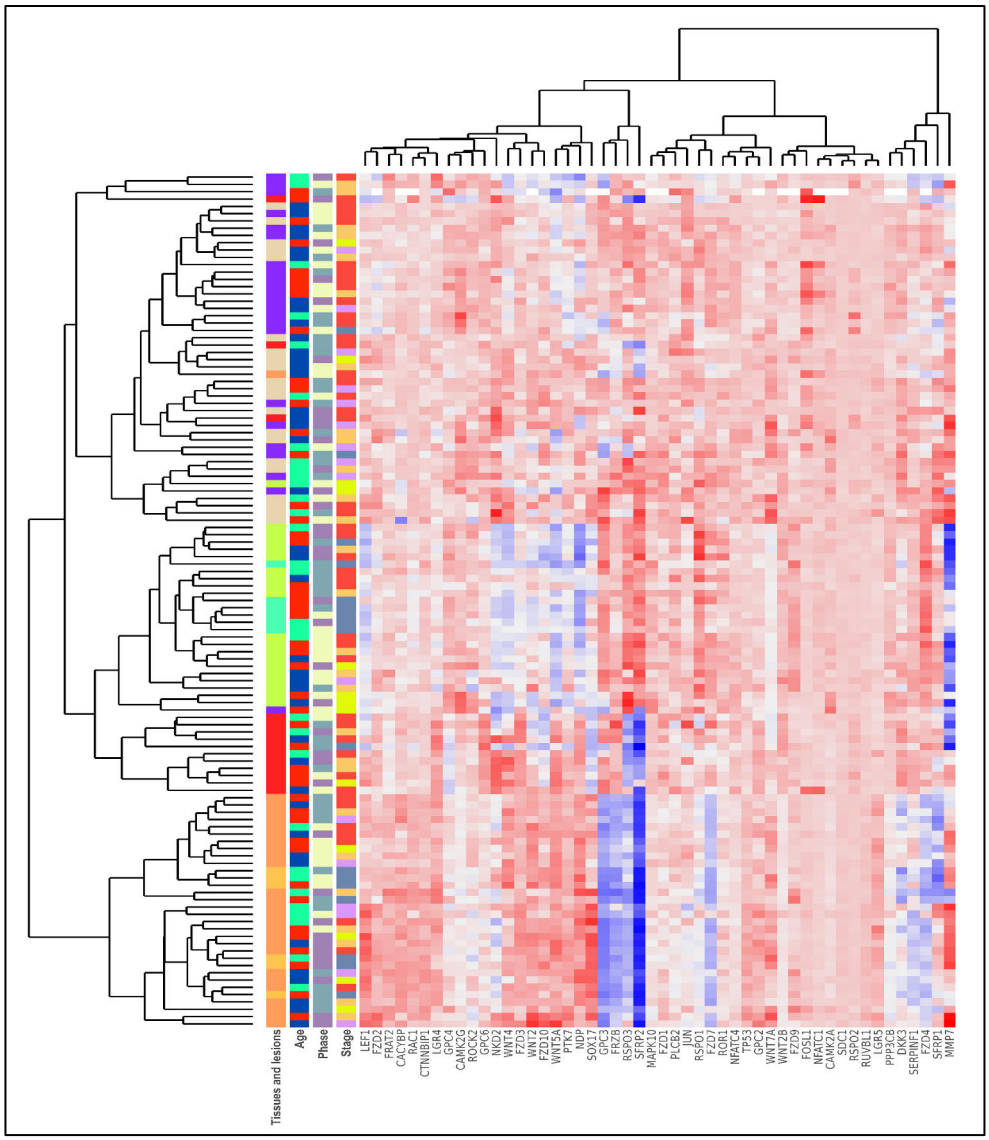


Figure 18. Example output of unsupervised hierarchical clustering analysis of differentially expressed WNT pathway genes in the endometrium, peritoneum and endometriosis lesions generated from the EndometDB GUI [602] as reported by Heinosalo *et al*. 2018[634]. The different clinical features of the samples (lesion/tissue type, age of the patients with pre-selected grouping, hormonal stage, and disease stage) are attached to the heatmap. Manhattan distance metric with complete linkage clustering method was used showing clusters corresponding to lesions and tissue types. The dendrogram on the x-axis shows the hierarchical relationship between the tissues and lesion as well as the cycle phase and disease stage. While the dendrogram on the y-axis shows the measure of similarities in the activation levels of the genes.

The correlation heatmap feature in the EndometDB analysis can be used with the most common correlation methods such as Pearson; Spearman; and Kendall, to show the correlation matrix between two discrete dimensions. The correlation heatmap (***I****, Fig. 4*; **Figure 19**) can be used to analyze how genes of interest correlate with each other in the different tissues and lesions with the most used hierarchical clustering methods. These methods provide information on the involvement of analyzed genes in the connected biological process.

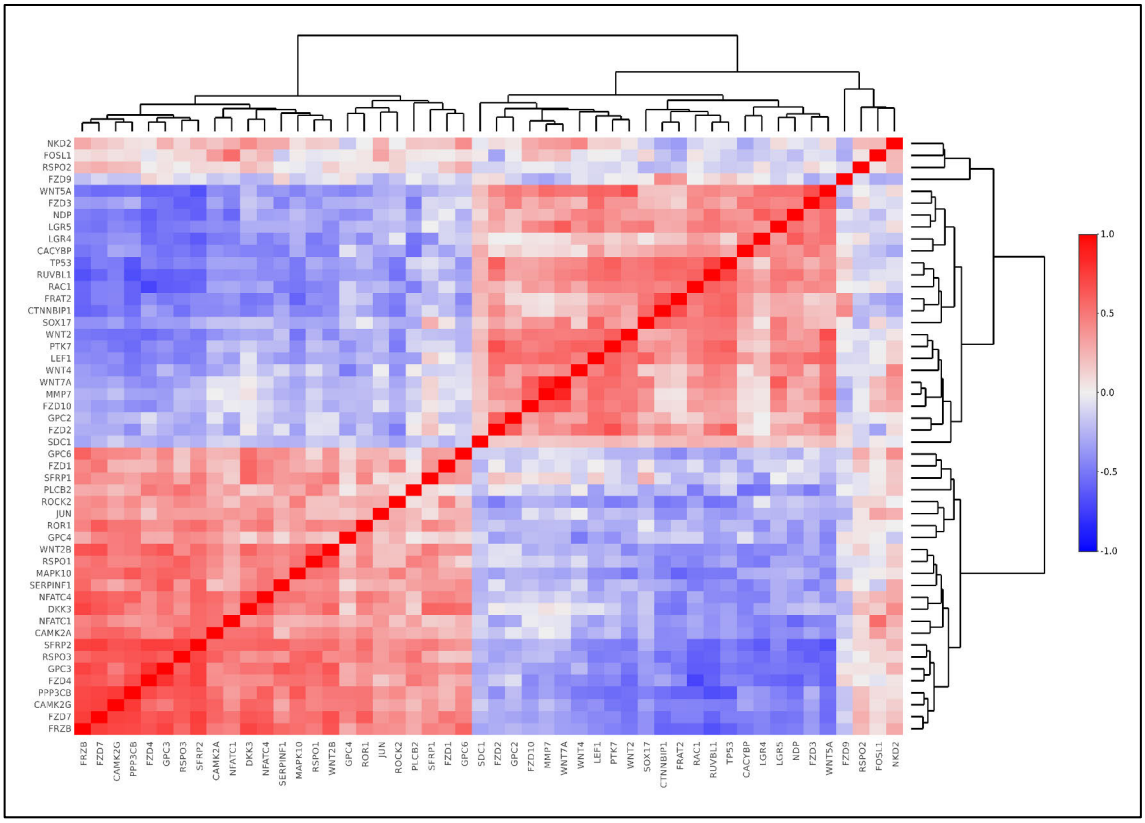


Figure 19. Correlation heatmap of WNT pathway genes with Spearman correlation method and complete linkage used as the clustering method generated from the EndometDB GUI [602].

Asides from the analysis plots the EndometDB includes three dimensionality reduction methods: Principal Component Analysis (PCA) (***I****, Fig. 5a*; **Figure 20**), Local Fisher Discriminant Analysis (LFDA) (***I****, Fig. 5b*; **Figure 21**), and Multidimensional scaling (MDS) (**Figure 22**) that can be used to further investigate similarities in the gene expression, between various sample types or to identify gene clusters to generate further hypotheses.

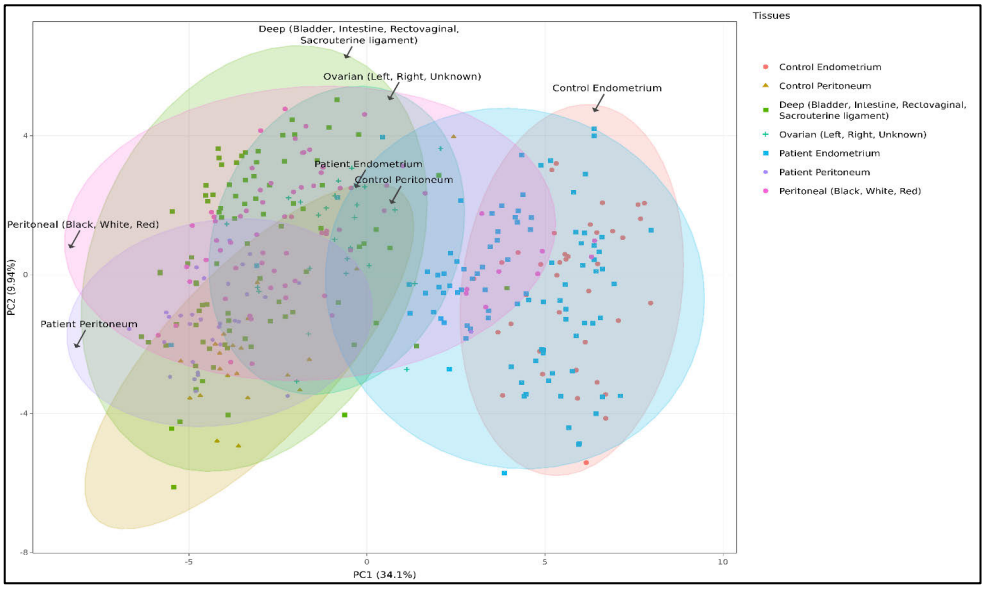


Figure 20. PCA analysis of the differentially expressed WNT pathway genes in the endometrium, peritoneum and endometriosis lesions colored by tissue types, and the confidence ellipses with 95% confidence level for the expression in various tissue types generated using the EndometDB GUI [602].

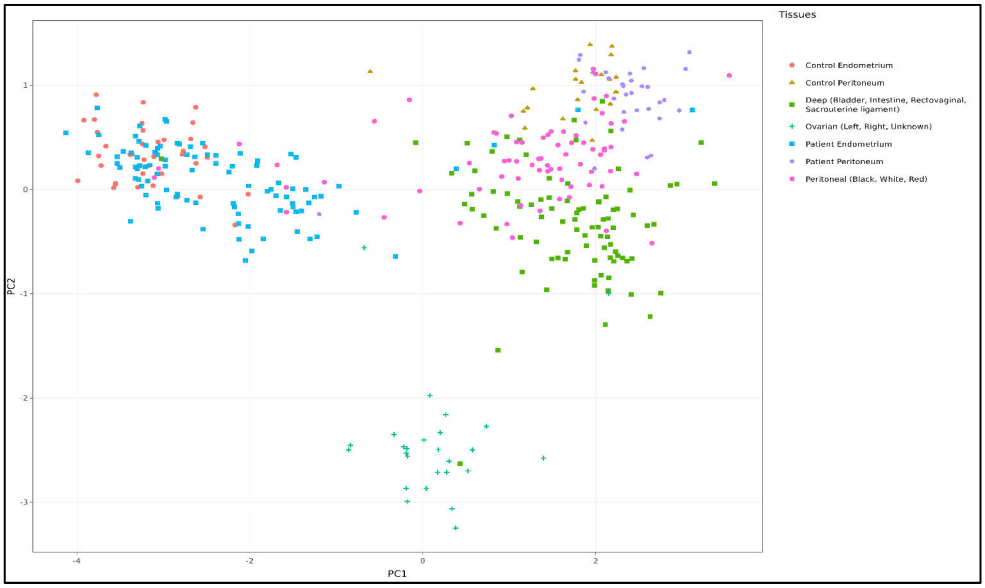


Figure 21. LFDA analysis of WNT pathway genes with orthonormalized metric colored by tissue generated from the EndometDB GUI [602].

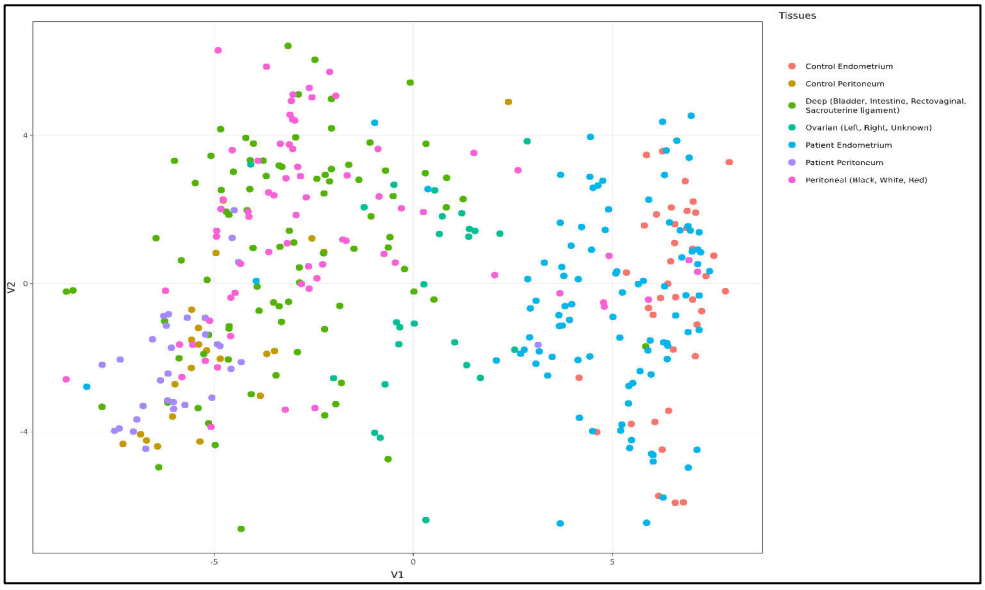


Figure 22. MDS projection output generated from the EndometDB GUI [602] of differentially expressed WNT pathway genes in the endometrium, peritoneum and endometriosis lesions colored by tissues/lesion with Euclidean used for the distance metric.

5.3 WNT Signaling in Endometriosis (II)

WNT (wingless-type MMTV integration site family) signaling is one of the most significant developmental signaling pathways that regulates cell fate determinations and tissue modeling during early embryonic and later development. It is triggered by very well preserved WNT proteins produced as palmitoylated glycoproteins and act like morphogens to form a concentration gradient across developing tissues [635]. Several studies, have suggested that the abnormal activation of the WNT/β-catenin pathway may be involved in the pathophysiology of endometriosis [379,636–642]. In addition, there is significant evidence of the role that WNT signaling in endometrium and endometrial diseases [313,378–380,636,638,639,641–646]. In II we used our, well-characterized cohort of human endometriosis samples from the Endomet database to analyze the expression of WNT signaling molecules in the endometrium of healthy women and endometriosis patients, as well as in ovarian endometrioma, superficial peritoneal endometriotic lesions and deep infiltrating endometriosis lesions. In global gene expression profiling, the WNT signaling pathway gene(s) were shown to be one of the significant pathways modified in endometriotic tissues, specifically, SFRP2 which was shown to be upregulated in both the superficial peritoneal and deep endometriosis lesions.

5.3.1 Alterations in WNT pathway genes expression in endometriosis in comparison with the endometrium

Analysis of the eutopic endometrium of healthy control women and endometriosis patients, and the different types of endometriosis lesions revealed strong changes in the WNT signaling pathway gene expression profile in the endometriotic lesions as compared to the eutopic endometrium of both healthy controls and patients. Eighty-five percent of the pathway genes listed in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and selected based on literature analysis were differentially expressed in at least one of the following comparisons: eutopic endometrium as compared with endometriosis, eutopic endometrium as compared with extraovarian endometriosis, eutopic endometrium as compared with ovarian endometriosis or ovarian endometriosis as compared with extraovarian endometriosis ($p < 0.05$) list of the significantly changed WNT pathway genes ($P < 0.05$, $FC \geq 1.4$) is shown in ***II**, Supplemental Table SII*. Hierarchical clustering analysis based on the mRNA expression of WNT signaling genes, revealed two well-defined clusters (***II** Fig. 1A*) indicating, there are major differences in the WNT pathway gene expression between both the eutopic endometrium and ectopic endometrium. Further clustering analysis showed cycle-dependent regulation of WNT pathway genes in the endometrium. However, this cycle-dependency was entirely lost in the endometriotic tissues, with further analysis showing that ovarian and extraovarian endometriosis differ in their WNT gene expressions. We analyzed a set of WNT pathway genes with mRNA expression using quantitative real-time polymerase chain reaction (***II**, Fig. 2*; **Figure 23**) to validate the gene expression changes identified from the global microarray data. The expression changes identified were like those observed with the microarray profile with statistically significant changes between the eutopic and ectopic endometrium (***II**, Supplemental Table SII*). Among the identified genes, SFRP2 gene coding was recognized as one of the highly upregulated genes in extraovarian endometriosis as compared with endometrium, both with the microarray and qRT-PCR. The expression of SFRP2 with qRT-PCR analysis was 183.3-fold higher in the extraovarian endometriosis than in the endometrium ($p < 0.001$), and in ovarian endometriosis, there was a 5.4-fold increase however there was no notable difference in the SFRP2 expression observed between the endometrium of healthy control women and endometriosis patients.

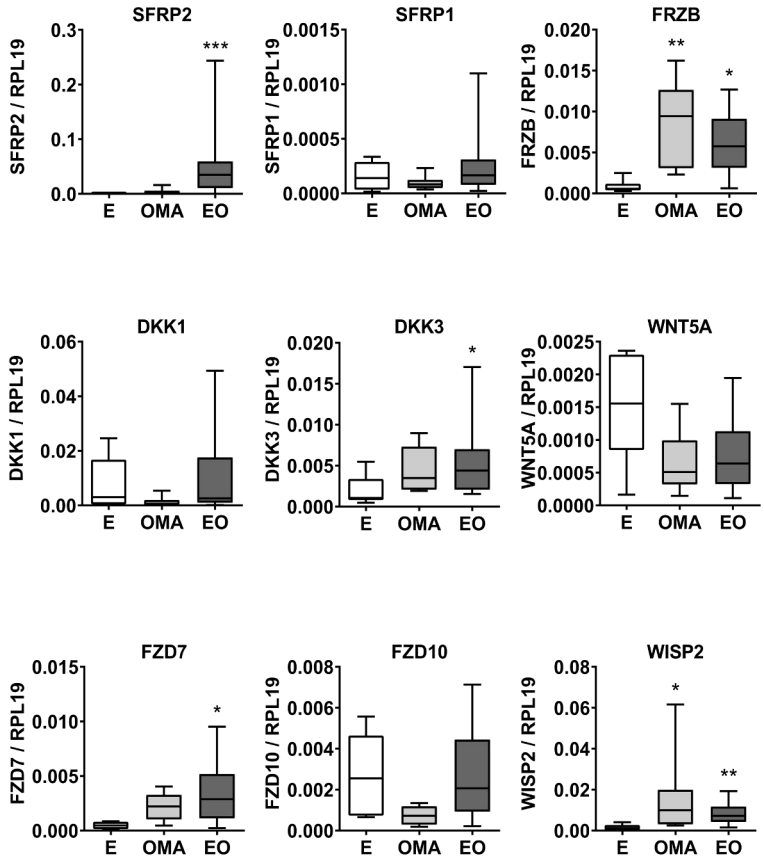


Figure 23. The expression of *SFRP1, SFRP2, FRZB, DKK1, DKK3, WNT5A, FZD7, FZD10* and *WISP2* analyzed using qRT-PCR to validate the data obtained by gene expression profiling. Statistically significant expression changes were seen *for SFRP2, FRZB, DKK3, FZD7 and WISP2. SFRP2* showed the highest expression increase between endometrium and extra-ovarian endometriosis (183.3-fold increase in extra-ovarian endometriosis), while the increment was much less (5.4-fold) for ovarian endometriosis. *E = endometrium, OMA = ovarian endometrioma, EO = extra-ovarian endometriosis, * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

5.3.2 SFRP2 defines lesion borders in extraovarian endometriosis

Western blot analysis from tissue homogenates showed significant increase in the expression of SFRP2 in extraovarian endometriosis as compared with ovarian endometriosis lesions and the patient endometrium ($p < 0.01$) (***II**, Fig. 3A and B*; **Figure 24 a** and **d**). Comprehensive analysis of cell specific expression during menstrual cycle was performed using IHC, with the data showing a high expression of SFRP2 in both epithelial and stromal cell components. In the endometrium of both the healthy controls and the patient a strong expression of SFRP2 was observed in

both the epithelial and stromal cells during the proliferative phase of the menstrual cycle but not in the secretory phase, while there was reduced SFRP2 expression in the epithelial cells of the endometrium, signifying a cycle-dependent suppression of SFRP2 protein expression in the endometrial epithelial cells (***II**, Fig. 3C, E and F*; **Figure 24 b**, and **e**). However, in extraovarian endometriosis, throughout the menstrual cycle there was no cycle-dependent downregulation of SFRP2 in the epithelial cells as we observed strong SFRP2 protein expression in both the epithelial and stromal components (***II**, Fig. 3D, G and H*; **Figure 24 c** and **f**).

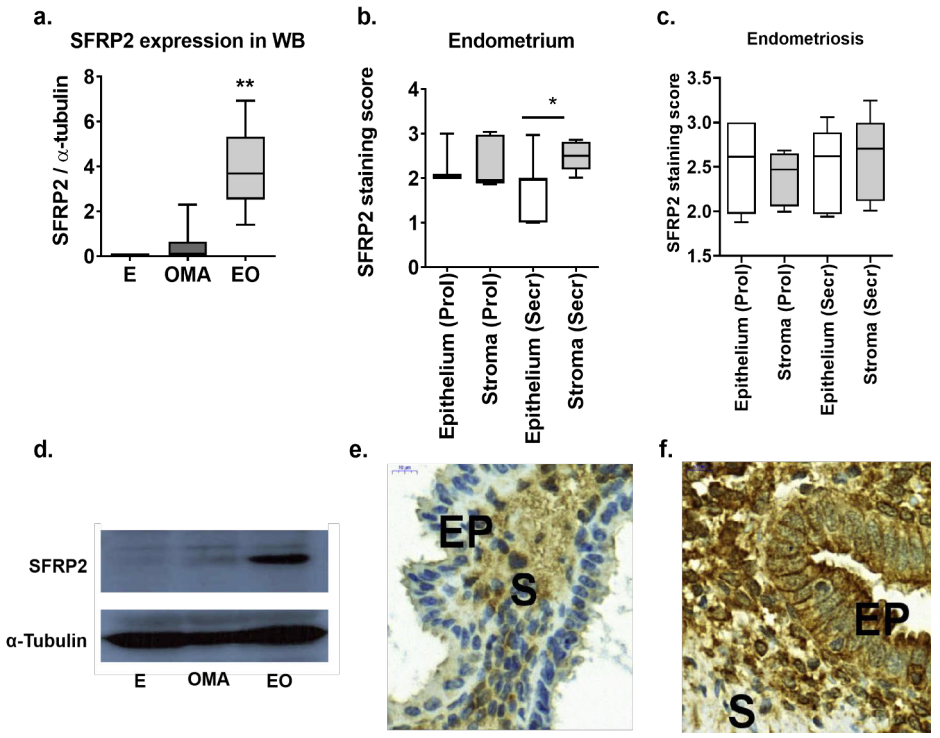


Figure 24. SFRP2 protein expression in endometrium and endometriosis. (a) A plot of protein intensities measured in the *E = endometrium, OMA = ovarian endometrioma, EO = extra-ovarian endometriosis*, showing SFRP2 expression normalized with α-tubulin expression. (b) A more detailed immunohistochemical analysis showed that in the endometrium, SFRP2 was downregulated during the secretory phase in the epithelium, but not in endometriosis (c). (d) A Representative Western blot, showing increased SFRP2 protein expression in extra-ovarian endometriosis. (e, f) Representative pictures at x100 magnification from the secretory phase of endometrium with apparent epithelial SFRP2 downregulation. In extraovarian endometriosis, equal staining in both the epithelium and stroma was observed in the secretory phase.

IHC and immunofluorescence of SFRP2 protein showed marked differences between the extraovarian endometriosis lesions and the normal tissue surrounding the endometriosis lesion area (***II**, Fig. 4A - H*). As β-catenin (CTNNB1) is a key

mediator of the canonical WNT pathway, we analyzed the expression pattern of β-catenin in extraovarian endometriosis, and similar expression patterns were detected for both SFRP2 and β-catenin (***II***, *Fig. 4A - H*). Both SFRP2 and β-catenin were found to have high protein expression in the same region as CD10, CD10 being an immunohistochemical marker currently used in identifying endometrium stroma surrounding the epithelium of endometriosis lesions [647]. Unlike with CD10, high protein expression of SFRP2 and β-catenin were also found in the epithelium and with low expression in and around the lesion border (***II***, *Fig. 4D*).

Analysis projected two strong progesterone response elements (PREs) in SFRP2 promoter to be present (***II***, *Fig. 3J and K*). We, therefore correlated the intra-tissue steroid hormone data [648,649] with SFRP2 gene expression profiles, and identified negative correlation between SFRP2 expression and intra-tissue progesterone concentration in extraovarian endometriosis ($r = -0.552$, $p < 0.05$), with no correlation found with estradiol and testosterone. Furthermore, using clinical data from questionnaires collected at the day of surgery we observed positive correlation between SFRP2 expression and abdominal menstrual pain symptoms occurrence ($r = 0.300$, $p < 0.05$), while no or very weak correlations with SFRP2 expression were found for other symptoms and patient characteristics ($r = 0.3$), as shown in ***II***, *Table III and Fig. 3L*.

5.3.3 Increased β-catenin protein expression in extraovarian endometriosis

We examined the subcellular localization of β-catenin in the endometrium and extraovarian endometriosis with immunohistochemical analysis. The IHC analysis revealed considerably more nuclear staining in both the epithelium and stroma of endometriosis as compared with the endometrium (***II***, *Fig. 5A-F*), as well as increased membranous staining in extra-ovarian endometriosis suggesting the canonical WNT signaling pathway is more active in extraovarian endometriosis than in the endometrium. To further determine the activation of the canonical WNT signaling pathway in endometriosis, we analyzed the expression of known human β-catenin target genes. Twenty-seven of the fifty-four human β-catenin target genes upregulated upon β-catenin activation were differentially expressed in extraovarian endometriosis compared with control and patient endometrium, further strengthening the hypothesis of increased activation of the canonical WNT pathway in extraovarian endometriosis. The expression patterns (analyzed using microarray) for the selected β-catenin target genes are shown in ***II***, *Fig. 5G*. A more comprehensive analysis of the hierarchical clustering pattern of the human β-catenin target genes revealed similar clusters observed when analyzing the WNT pathway genes (***II***, *Supplemental Fig. S2*). The endometrium and endometriosis clustered

separately, with ovarian and extra-ovarian endometriosis also forming separate clusters. Of the 8 β-catenin target gene shown in ***II**, Fig. 5G*; **Figure 25**, three of the genes (CLDN1, JUN and VEGFB) were differentially expressed in the proliferative phase compared to the secretory phase, with no cycle dependent regulation in endometriosis. The full list of upregulated targets with their microarray expression differences is shown in ***II**, Supplemental Table SIII*.

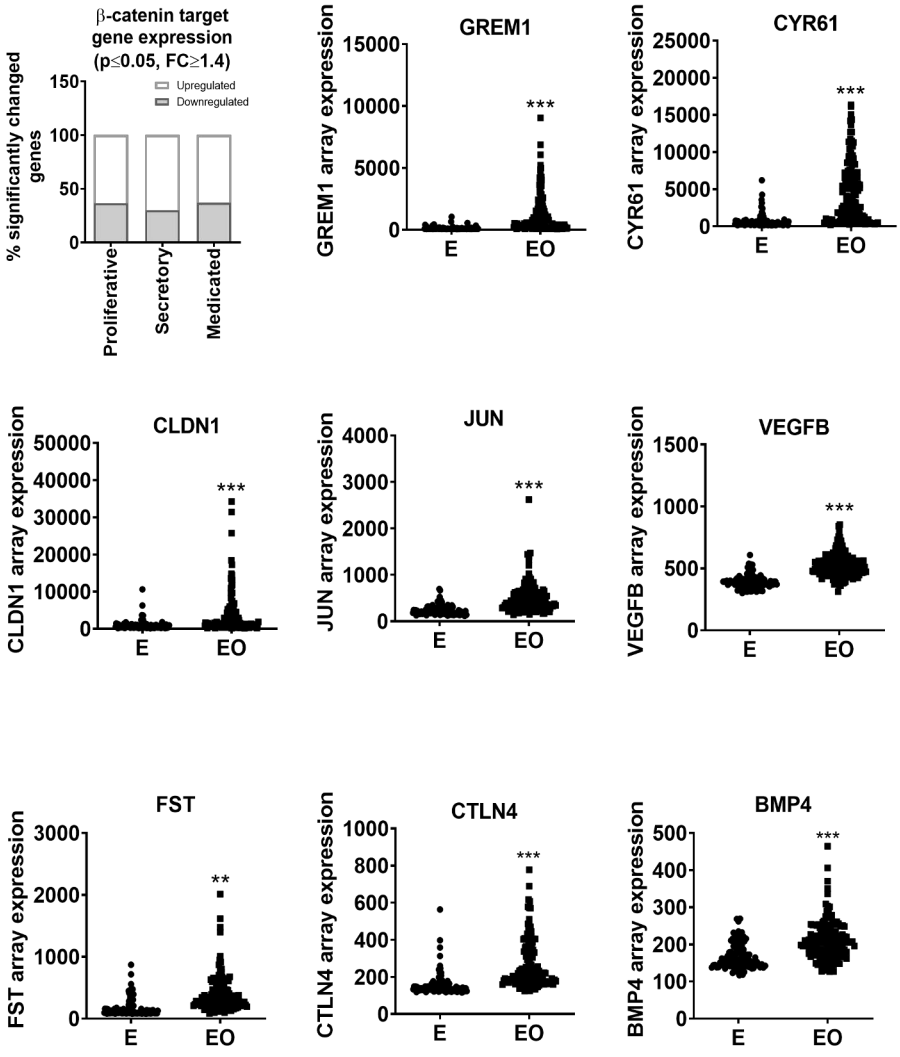


Figure 25. β-catenin target gene expression analysis showing upregulation of various β-catenin target genes as compared with endometrium. Of the significantly changed targets 67 % were upregulated ($p \leq 0.05$, FC ≥ 1.4). ** $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

5.3.4 SFRP2 is a WNT signaling agonist in endometriosis, and it regulates the canonical β-catenin mediated signaling pathway

As SFRP2 has been shown to act as a WNT signaling agonist or antagonist, depending on the tissue environment, we examined the function of highly expressed SFRP2 in extraovarian endometriosis. Primary cultured extraovarian endometriotic cells from human lesions were exposed to SFRP2 siRNA-mediated knockdown, and measured cell proliferation. The SFRP2 mRNA expression was decreased by 72 % ($p < 0.05$), and protein expression reduced by 60 % ($p < 0.01$) as compared with the control siRNA (***II****, Fig. 6A-C*). SFRP2 knockdown resulted in a significantly reduced cell proliferation (48 %, $p \leq 0.05$) *in vitro* (***II****, Fig. 6D*), and cell proliferation and SFRP2 mRNA expression showed strong positive correlation in all samples in the siRNA experiment ($r = 0.732$, $p < 0.01$) (***II****, Fig. 6G*). Furthermore, Western blot analysis showed an average of 33.1 % reduction in total β-catenin protein expression ($p < 0.05$) after SFRP2 knockdown as compared with treatment with the non-targeting siRNA (***II****, Fig. 6E, F*), suggesting a role for the canonical WNT/ β-catenin pathway in the reduction of cell proliferation and regulation of extraovarian endometriosis growth.

5.4 Description of the predictive model (III)

The Endomet database structure and content has allowed us to create a symptom-based predictive model for risk assessment and early prediction of endometriosis among women. We used several advanced machine learning methods described in the methods section to combine clinical features from questionnaires with serum biomarkers. The questionnaires were designed in agreement with the World Endometriosis Research Foundation (WERF) and the European Society of Human Reproduction and Embryology (ESHRE) guidelines. The multi-dimensional data analyzed using machine learning algorithms enabled the identification of multiple sets of features that effectively characterize distinct pathways and identify disease classes. We used predictive models in supervised and semi-supervised learning framework to extract patterns that are predictive for disease risk, progression, and recurrence with histopathological data used as class labels in the training and to assess the performance of the predictive models. The machine learning process used in developing and training the risk assessment model for endometriosis diagnosis using clinical symptoms and serum biomarkers shown in **Figure 26**, ***III****, Fig. 2*.

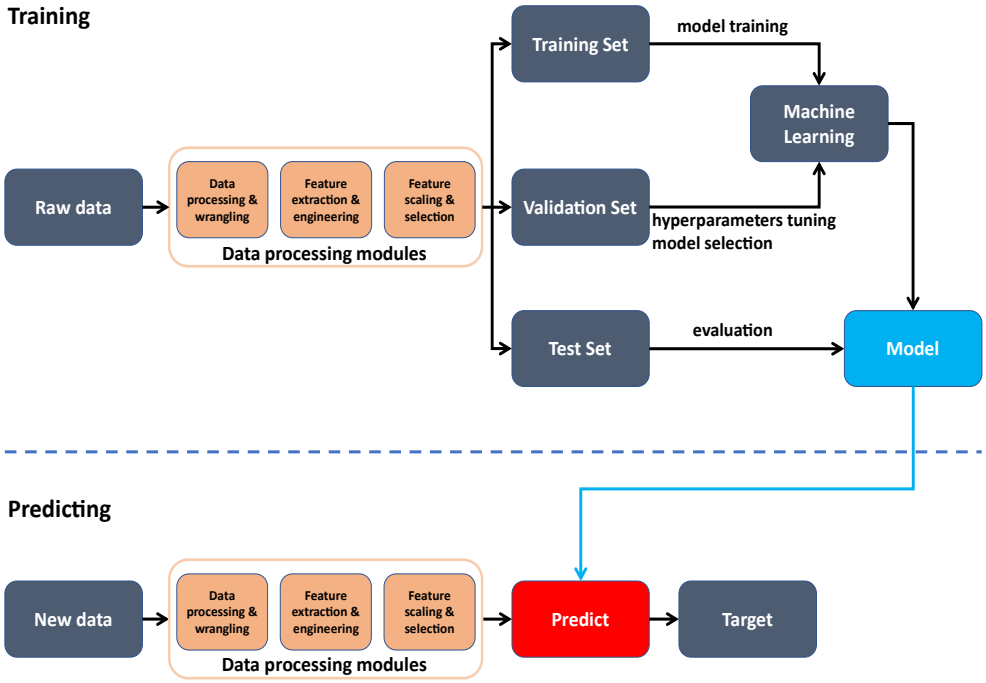


Figure 26. Machine learning process used in developing and training the risk assessment model for endometriosis diagnosis.

5.5 Model generation and development (III)

The predictive model was developed in 3 stages: in stage 1 only patient characteristics with symptoms which we categorized as clinical features were used in developing the model. To select the best discriminating clinical features, we used wrapper feature subset selection (recursive feature elimination) method [625,626] in a repeated CV setting and ensemble the results across different repeats of the CV. The results from the repeated cross-validations were compiled into a final list of features to be used for classification. Once the most discriminating features had been selected, nested five-fold CV was used to train our machine learning classifier for the final classification as shown in **Figure 27** with RF used as the machine learning classifier [627].

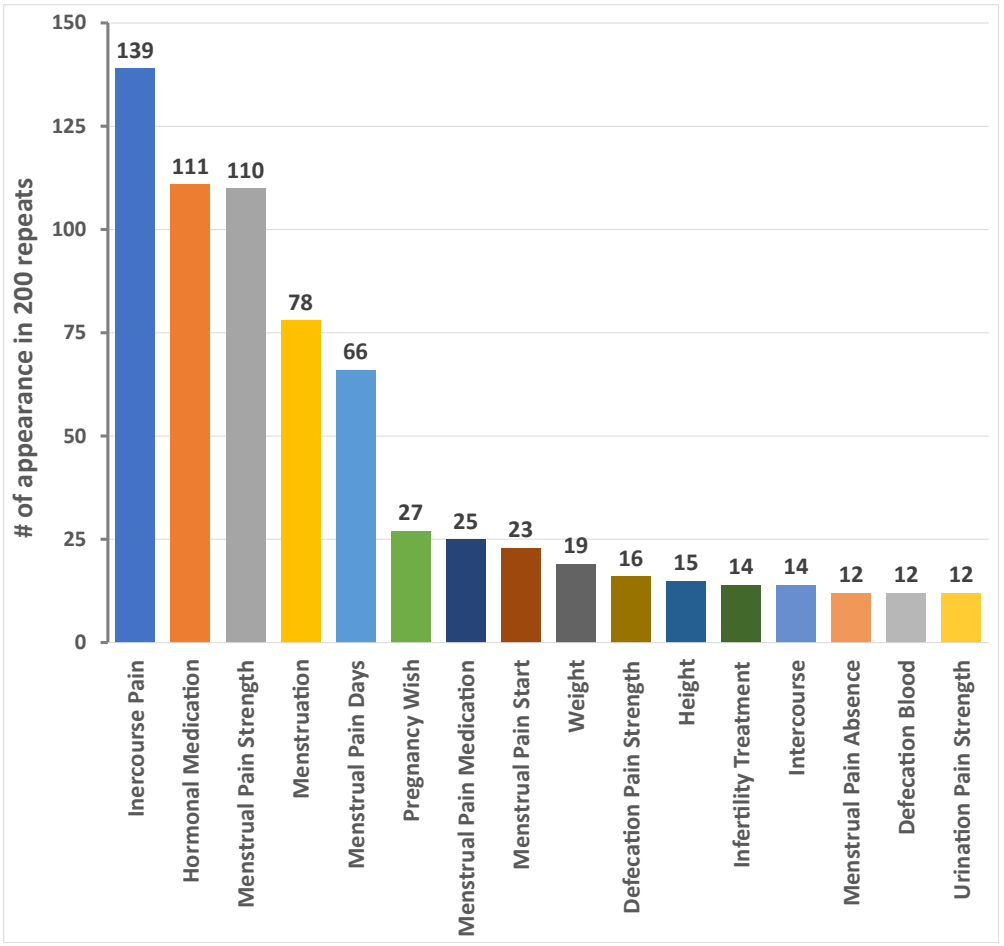


Figure 27. Most predictive clinical features. Clinical data in disease prediction. Final classification with decision tree and nested 5-fold cross-validation in 200 repeats.

In stage 2 we used only biomarkers in the predictive model. The same wrapper feature subset selection method was employed to selecting the best discriminating biomarkers from all metabolites, cytokines, serum hormones concentration, CA-125, HE4 and 6 cancer markers. The result put to a list of features used in classification. We then used nested fivefold CV to train the machine learning classifier for the final classification on the selected list of features as shown in **Figure 28**.

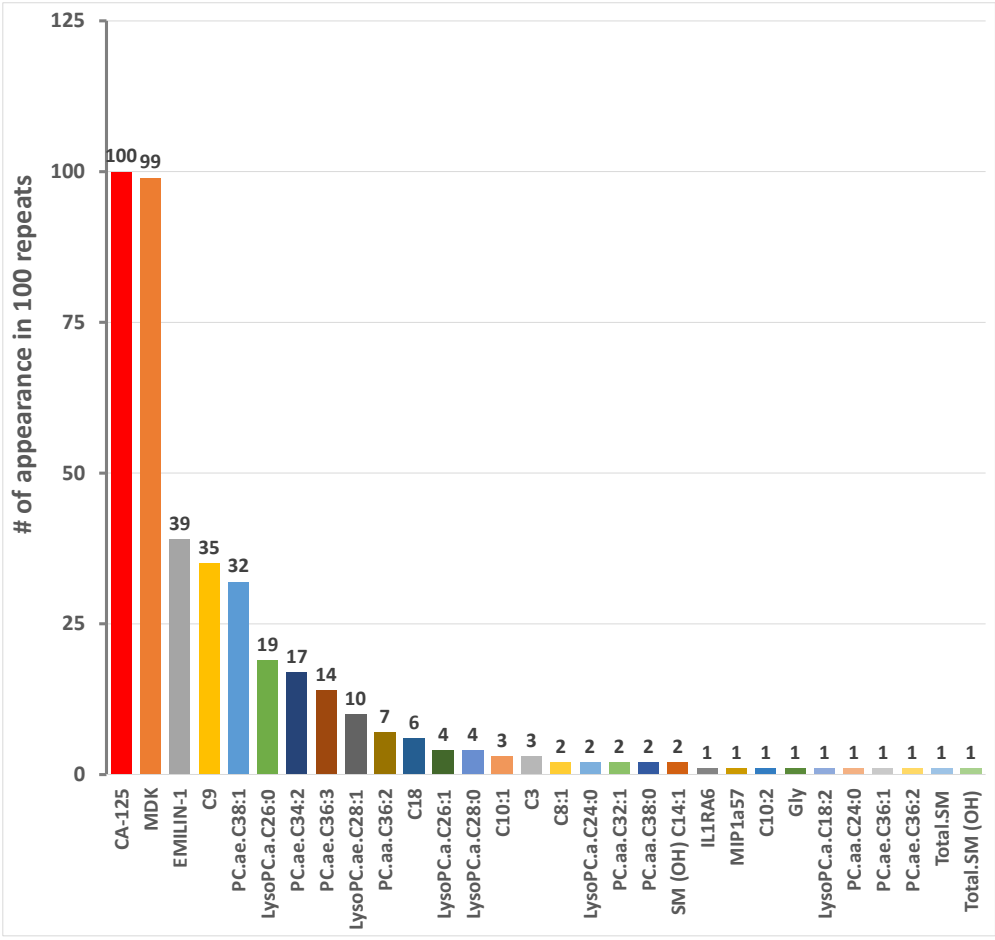


Figure 28. Biomarkers only. Metabolites, cytokine, serum hormone concentration, CA-125, HE4, and 6 cancer markers. Final classification with decision tree and nested 5-fold cross-validation in 100 repeats.

In stage 3 we combined both clinical features and biomarkers in the predictive model. After combining clinical features and biomarkers, we used the same recursive feature elimination methodology [625,626] used in the previous models to select the best discriminating clinical features and biomarkers in a repeated CV setting and ensemble the results across different repeats of the cross-validation (***III***, *Fig. 3*). In feature selection, we used 100 repeats of cross-validation. We based the predictive models on personalized biomarker signatures and clinical features and adjusted for confounding factors, such as age, BMI, and medication status. We then trained the predictive algorithms (filtered sets of decision trees) to identify patients with high risk of endometriosis.

5.6 Validation of the predictive model (III)

To demonstrate the predictive performance of the chosen model, we trained the final model on the full data from cohort I, and the cohort II was used to validate the generalizability. Cohort II as a validation cohort eliminates the risk of reporting overly optimistic results due to the potential for sample bias and overfitting in ML models resulting in better representation when predicting risk in future unseen cases. The performance of the 3 stage models in predicting endometriosis was assessed by analyzing the receiver operating characteristic (ROC) curve. Model sensitivity, specificity, and positive and negative likelihood ratios were also calculated, and the best model cut-off points considered to be those corresponding to the highest sum of specificity and sensitivity. Without reassessment, the model produces acceptable results for cohort II as shown in **Figure 29**; ***III***, *Fig. 6*.

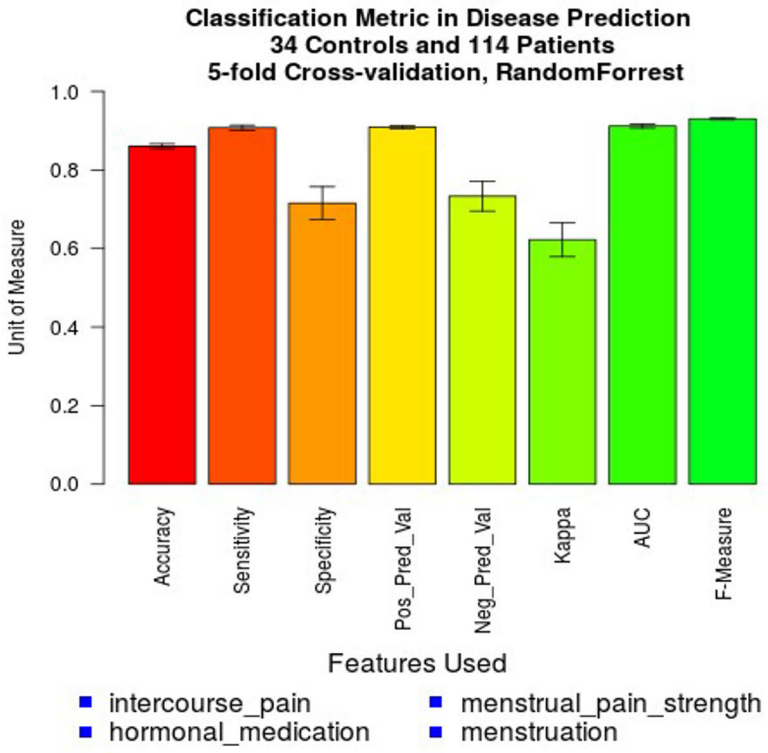


Figure 29. Predictive prowess of using only clinical features.

The cross-validation sensitivity and specificity of endometriosis diagnosis first stage analyses when using only patient characteristics and symptom with the most predictive features was 0.95 and 0.85 respectively, with an AUC of 94% (***III***, *Fig.*

5). When only using serum biomarkers the sensitivity and specificity was 0.88 and 0.78 (***III****, Fig. 5),* the nested cross validated accuracy of the model to diagnose endometriosis was between 82% and 93%, respectively, with an AUC of between 87% - 95% depending on biomarker combination (***III****, Fig. 4*). And when symptoms and patient characteristics are combined with the most robust serum biomarkers, the cross-validation sensitivity and specificity was 0.94 and 0.91, respectively, with an AUC of 98% (***III****, Fig. 5)*.

The independent sensitivity and specificity in the validation cohort (cohort II) when using both clinical features with serum biomarkers was 1.0 and 0.80, respectively, with and AUC of 0.96 (***III****, Fig. 6*).

6 Discussion

Our current understanding of the pathogenesis and etiology of endometriosis is still quite limited, even though for several decades there has been significant efforts dedicated to their study. There remain several important unresolved questions. Among them, what is the origin of the different endometriosis phenotypes? Why do lesions with normal appearing histology always induce chronic inflammation, while their uterine equivalents do not? Why does DIE lesions behave as disseminated cancer? [650] What is the origin of pain? How do we reduce diagnostic delay? as well as how to choose individual treatment options? Since its characterization, a century ago, researchers, clinicians, and patients alike have mulled over these questions. In assessing the experience of patients with endometriosis, pain and infertility are 2 of the most common symptoms and typically of greatest concern. However, the overall toll is much greater as these women experience diminished quality of life, increased incidence of depression, adverse effects on intimate relationships, limitations on participation in daily activities, reduced social activity, loss of productivity and associated income, increased risk of chronic disease, and significant direct and indirect healthcare costs [52,651–655]. Recommendations were updated recently for future research priorities in endometriosis to include the development, validation, and implementation of new endometriosis classification systems as well as the discovery of accurate non-invasive diagnostic methods combined with clinical characteristics to improve diagnosis [218].

6.1 Challenges and limitations of current endometriosis diagnosis

There are no pathognomonic features in the clinical presentation or biomarkers necessary and adequate to define endometriosis. Instead, there are key symptoms that prompt surgical evaluation, such as pain and infertility, which can have multiple alternative causes [52]. Endometriosis is difficult to diagnose for several reasons. One of the factors is insufficient understanding of the disease by health-care professionals. It is typically defined by its histological evaluation and further described based on location and depth of lesions. However, the presence of lesions does not in any way exclude other etiologies for the symptom's patient experience,

and the absence of obvious lesions does not eliminate the possibilities of endometriosis. Moreover, there are poor correlations between symptoms and disease severity or extent, as measured by existing staging systems [9,52]. The current diagnostic model, necessitates laparoscopy with or without histologic verification as the gold standard, despite the fact that many societies endorse the management of symptoms prior to obtaining a definitive surgical diagnosis [3,52,83,84,105,221] except in cases where fertility is a priority [656]. The advantages of laparoscopy and its role in management must not be underestimated, however its precision, risk, and cost effectiveness merit reassessment. The inadequate relationship between symptoms and the extent of disease discovered at laparoscopy demonstrates the shortcomings of surgical disease evaluation [52,205].

Asymptomatic endometriosis [657–659], the fortuitous finding of endometriotic foci during other laparoscopy surgeries, e.g. tubal ligation [27,71], confounds and further challenges our understanding of the connection between the existence of endometriotic foci and the prevalence of pelvic pain in symptomatic women [52].

The dependence on laparoscopy for endometriosis diagnosis strengthens the perspective that the existence of identifiable lesions in the pelvis is the main principle of endometriosis, instead of approaching endometriosis as a menstrual cycle dependent, chronic, inflammatory, systematic disease that frequently presents as pelvic pain [52]. By changing the focus to the patients instead of the lesion, the clinical diagnosis has the possibility to be more comprehensive with reduced diagnostic delay. Diagnosing endometriosis using nonsurgical methods has been reported to reduce the mean time from the appearance of symptoms to when a definite diagnosis is obtained compared to insisting on surgical diagnosis or verification [660]. This change, nevertheless, requires reliable clinical diagnostic methods to accurately detect endometriosis.

Even with increasing awareness and apparent interest in endometriosis, the diagnostic delay has not been shown to be shortened. [661,662] Primarily the delays could stem from reluctance to seek medical help, especially in adolescent. Early age considerably increases the delays and often the first indications of endometriosis have their debut in teenage years shortly after the menarche [663]. Additionally, symptoms occurring throughout the adolescent period might be overlooked by healthcare professionals. The stigma associated with menstrual issues and the social normalization of women's pains often considered to be a normal pattern or misinterpreted is another cause for delays. One study discovered that women wait on average of 2.3 years from the beginning of symptoms before seeking assistance [661]. The implications may include social isolation, poor self-esteem in a vulnerable period in their young life with long-lasting consequences.

Although prevalent amongst women with endometriosis, pelvic pain alone is inadequate as an indicator of endometriosis, as it may be linked with multiple

gynecologic and non-gynecologic conditions which may have pelvic pain in their symptomology [78,241]. Additionally, pelvic pain is not present in all endometriosis patients and when present women may not understand their pain as a treatable condition, particularly if this pain started already at menarche [664]. Nevertheless, pelvic pain characterized as cyclic, chronic, and progressive or persistent increases the probability of an association with endometriosis [73–75]. Pain is characteristically menstrual (dysmenorrhea), although may progress to include non-menstrual pelvic pain, common amongst patients diagnosed with endometriosis [665]. In a quantitative study, participant characterized their pain symptoms as severe and progressive throughout both menstrual and non-menstrual phases in response to questions about their experiences living with endometriosis [653]. Along with pain endometriosis patients were much more likely to report dyschezia, dysuria, and dyspareunia, than unaffected women [41,73,74,242,251,254]. While the sensitivity of dyspareunia is usually low, [244,247,666] its presence is not particular to endometriosis, deep dyspareunia on the other hand is associated with deep endometriosis [254].

6.2 Databases in scientific and medical research

Precise and accurate data are equally valuable resource for as well as essential outputs from scientific research. It is through the creation of ideas, communication, and the use of facts that scientists conduct their research. Throughout the history of science, new findings and ideas have been documented and used as the foundation to further scientific advancements and for educating others. Because of the near complete digitalization of data collection, dissemination, and manipulation over the past decades, almost every aspect of the natural environment, human activities, and in fact every single life form can be observed as well as captured in digital form in a database [667]. There is hardly any sector of our lives that is not involved in the establishment and development of digital databases. Undoubtedly science is not an exception in its ever-increasing dependence on the exploitation and creation of databases.

The collection of vast quantities of clinical data has become more and more prevalent, and so are the scientific studies that utilize such data [668–670]. This is a fast-growing area, and these rich data sources provide a lot of potential benefits. The rapid evolution of information technology infrastructure, and the ability to store, manipulate, share, and process copious quantities of data, associated with the greater acknowledgement of the value of clinical data and the increase in the associated science, have driven this growth. Using '*big data*', a wide range of research questions with corresponding research studies and design can be conducted. There are advantages to utilizing such data sources such as their relatively substantial number of patients and comprehensive nature, allowing subgroups and rare events

to be investigated. Although expensive to setup and maintain once the infrastructure is in place the database provides a platform that allows for numerous resource and cost-efficient research studies compared to studies using bespoke data collection.

In study **I** we developed the Endomet database to allow for further characterization of specific pathways involved in endometriosis using gene expression profiles. There is still a great need for a more systemic and thorough analysis of the expression patterns of genes across different types of lesions because the different forms of endometriosis may express different markers/genes differently [261].

Analyzing the different lesion types could aid in the identification of potential diversity within the etiology of the different lesions. As an illustration of what is achievable with this data source, utilizing the data that contained in the EndometDB we identified Secreted frizzled-related protein 2 (SFRP2) in study **II** to be a gene with relative high expression in endometriosis in comparison with the endometrium. The protein was shown to be a novel lesion border marker in histological sections, and as a secretory protein it has a potential to serve also as a serum biomarker [634].

The EndometDB in study **I** integrates clinical data (***I****, Fig. 1a*) and tissue types (endometrium, peritoneum, and the different endometriosis lesion types) with transcriptomic data (>48000 measured). This enables the exploration of the data through various patient factors, such as age, cycle phase, disease stage and hormonal medication status. The EndometDB is a handy tool that can be used to identify potential biomarkers and treatment targets, and to gain new information on the gene expression networks linked with the lesion growth. The resource established is expected to contribute to the development of novel diagnostic and prognostic markers predictive of endometriosis and to understanding the pathogenesis of endometriosis better.

6.3 AI- big data – algorithms for research (etiology) in general and then endometriosis

The growth and intricacy of *big data* in healthcare implies that artificial intelligence (AI) will be used increasingly within the field of medicine. Multiple AI applications are currently being utilized by patients and healthcare providers, as well as life sciences companies. Many AI applications in healthcare consist of diagnosis and treatment recommendations, administrative activities, patient engagement, as well as adherence. Although there are numerous examples wherein AI can successfully conduct healthcare tasks as good or better than humans, ethical issues in the application of AI and implantation will prevent large-scale automation of healthcare.

Since at least the 1970s AI in healthcare has focused solely on diagnosis and treatment of disease when MYCIN was developed at Stanford for detecting bloodborne bacterial infections [671]. MYCIN and other early rule-based systems even

though demonstrated promise in correctly identifying and treating disease they were not adopted for clinical practice because they were not substantially better than specialists and were poorly integrated with existing workflows for clinician and medical record systems [672]. Even Though rule-based systems are widely integrated within electronic health record (EHR) systems, [673] they do not have the accuracy of more computational systems based on machine learning and autonomous decision-making. These rule-based decision support systems are hard to maintain as medical knowledge grows and changes, the systems are frequently not able to cope with the explosion of data and knowledge based on genomic, metabolic, proteomic, and other 'omics-based' methods used in healthcare [672].

The value of using AI tools is based apparently on the compromise between the potential benefit and associated risk as the benefits outweigh the risk, there is higher value placed on the use of technology. Studies have highlighted the significance of AI in healthcare, particularly in medical informatics [674] since it is able to offer enhanced patient care, improved diagnosis, and better interpretation of medical data [675]. In 2017 machine learning was utilized successfully to diagnose skin cancer as efficiently as dermatologists [544] with some claiming AI able to accomplish this task better than dermatologists [676]. In April of 2018, the Food and Drug Administration (FDA) authorized the very first AI device to diagnose diabetic retinopathy without help from a physician in the USA [677]. There is increasing investments in the development of AI embedded in health devices or applications to enhance patient safety, improve the quality of practice, and patient care management, as well as decrease healthcare costs.

6.3.1 AI and precision medicine

We have taken giant strides in our quest for answers, and in spite of all our scientific knowledge, much of medicine is still based on alleviation of symptoms and performing learned trials based on treatments, which works for most patients to reduce the risk of complications [678], bring symptom relief, as well as improve chances of survival, but not for everyone. To get new knowledge about etiology, pathogenesis, and disease categorization, it is essential to comprehend how diseases are linked to one another [678]. Intelligent big data platforms are critical to furthering our understanding of diseases with AI accelerating the investigation of hidden elements in clinical data and obtaining effective gap-based information about patients for the early identification and prevention of constitutional disorders [678]. During recent years, the concept of precision medicine using AI has developed into a fundamental creativity pillar for prominent research in the development of health care processes, and has great potential in patient care [679,680]. Precision medicine holds the potential to improve the symptom driven practice of medicine by intelligently

integrating multiple omics profiles with imaging, epidemiological, demographic and clinical details to allow for better and economical personalized treatment and a wide range of earlier interventions for advanced diagnostics and tailoring [678]. While complications associated with diseases on an individual level have made it challenging to make use of healthcare information alone in the clinical decision-making process, some of these existing limitations have been curtailed by innovations in technology [681]. As biotechnology has progressed enormously and computers have become faster in speed, heterogeneity and the growing volume of datasets have fueled the AI engine in discovering technological improvements to resolve complicated problems in science and medicine. Sufficient, diagnostic, and intelligent access to healthcare data has the potential to transform the field of medicine by improving outcomes and reducing cost, enhancing the quality and transition of care, detecting diseases in early stages and developing a better understanding of biological mechanism by modeling multifaceted biological interactions through an all-inclusive integration and analysis of knowledge [682–684].

6.4 AI and ML based algorithms in healthcare

6.4.1 Symptom and biomarker based predictive models in endometriosis

AI has contributed vastly to building an ecosystem in the healthcare sector, and there have been numerous attempts to use AI in the development of predictive models to assess the risk or predict endometriosis in women before surgical intervention [236–242,252–255,593–595,685]. Nonetheless, these attempts have not produced any simple viable solution that can aid decision making in clinical setting or reduce diagnostic delays, associated high healthcare costs, and the personal suffering in women with endometriosis related symptoms. In these studies, various statistical and modeling techniques such as logistic regression analysis, were used to analyze data obtained from relatively small sample numbers, although internal cross validation within the dataset was attempted in some of these studies [236,237,242,253,254]. In one such study, a classification tree correctly classified only 38% of non-ovarian endometriosis cases [594]. In others, the likelihood of finding deep infiltrating lesions was evaluated with varying results of success [593,595]. In another study, predictive models were generated to predict pregnancy rates following surgical diagnosis of endometriosis [242].

There have been no attempts so far to predict the presence of endometriosis using ensemble ML models that combine multiple predictors. In study **III** using data described in study **I** we used random forest (RF) based ensemble models in predicting endometriosis by combining both clinical and biomarker data. We developed a symptom and serum biomarker based predictive model for the risk

assessment and early prediction of endometriosis. The model was developed using both clinical features that includes symptoms and serum biomarker data obtained from women who were about to undergo laparoscopy or laparotomy for endometriosis associated pain symptoms, such as dysmenorrhea, dyspareunia, or pelvic pain. With the model validated in a separate cohort of women, we were able to achieve specificity and sensitivity, with a relatively high degree of confidence (***III**, Fig. 5*). The predictive power of the models was also evaluated using the area under receiver operating characteristic (AUC) curve in a cross-validated setting (see results). The predictive model equates and ranks endometriosis associated symptoms such as dysmenorrhea, dyspareunia, and dyschezia as symptoms with the strongest predictive performance in the model, in line with the fact that these symptoms are often associated with endometriosis related pain [686,687]. However, symptoms, such as abdominal pain, dysuria, chronic pelvic pain, infertility, and chronic fatigue did not fall into any significant category of predictors.

Among serum biomarkers used, CA-125 provided the strongest predictive value when predicting endometriosis. CA-125 measurement is considered an essential component in the diagnostic examination of women with adnexal mass [688]. Despite its shortcomings, It has been used extensively to detect and monitor the progression of endometriosis [685,689–693]. In epithelial ovarian cancer detection CA-125 has a set cutoff value of 35 U/mL [694], but in endometriosis screening a decisive cutoff value cannot be set as serum level may not necessarily correlate with the severity of endometriosis [695]. Other serum proteins that were shown to be of importance in the model when predicting endometriosis include EMILIN-1, MDK, IL-1Ra, EGF, and HE4. HE4 is a marker currently used to rule out ovarian epithelial cancer in patients with endometriosis related symptoms [696–698]. All these prognostic factors, except for HE4, showed significant difference in serum concentration levels between the patients and controls also when tested separately (***III**, Supplementary Table 4*). One notable serum biomarker that showed significant difference between the patient and control groups but was not considered an essential predictor in the ML model, was granulocyte colony-stimulating factor (G-CSF), this might be likely due to other features compensating for its predictive contribution in the model.

In clinical practice CA-125 remains widely used and is the only biomarker that has been reported recurrently to be elevated in endometriosis [699–703], and when included, in serum marker panels studied and in predictive models, CA-125 has constantly been among the best performing markers similar to our finding in study **III**. However, due to inadequate sensitivity in minimal-mild endometriosis, it is not considered reliable as a single marker in clinical practice [288,693,704–706] although widely used. Thus, a combination of multiple serum biomarkers and other features, such as the clinical features shown in the present study, are expected to offer more reliable prediction power.

6.5 The role of SFRP2 and WNT signaling in endometriosis

In study **II** we identified differentially marked activation of WNT signaling pathway components in endometriosis when compared with the endometrium, presenting evidence of a significant role for WNT pathway action in endometriosis growth. The highest expression change was detected for SFRP2 which is highly expressed in extraovarian endometriosis, but its expression less evident in ovarian endometriosis. The WNT signaling pathway is active throughout uterine growth and implantation, as well as during cyclic remodeling of the endometrium demonstrated by the fact that several WNT pathway elements are expressed in a cell-specific and cycle-dependent manner in the endometrium. In the proliferative phase of the menstrual cycle, WNT signaling is enhanced by estradiol, while in the secretory phase, inhibitory effect on WNT signaling by progesterone [707]. For individual WNT pathway genes, significant cycle-dependent differences have been previously detected [313,636,707–710], and sex steroids regulation has been shown for some of them [644,645,708,711–713]. Also, the expression of SFRP2 in the endometrium varied during the cycle so that the epithelial expression was downregulated in the secretory phase, and in study II we observed a significant negative correlation of SFRP2 expression with intratissue progesterone concentration in extraovarian endometriosis lesions.

Expression analysis of WNT pathway components and β-catenin target genes in study **II** showed clear clusters of endometrial samples separate from endometriosis with further analysis of the endometrium samples showing separate clusters for the proliferative and secretory phase. In the endometriosis samples there were no clusters observed according to the menstrual cycle phase, but there were further subclusters separating the ovarian and extraovarian endometriosis. In previous studies we have shown the cycle-dependent changes in estradiol and progesterone concentrations in the endometrium to be missing in endometriosis tissue and the loss of cycle phase -specific expression pattern of the WNT genes is likely to reflect the disturbed hormonal environment of endometriosis tissue [648,649]. Recent studies have shown that by binding to the estrogen-response element of β-catenin promoter in ovarian endometriosis estradiol directly upregulates β-catenin expression [644,645], which further supports our hypothesis that steroid hormone action is a central upstream regulatory mechanism of WNT signaling in the endometrium and endometriosis tissue.

Microarray data in study **II** from both ovarian and extraovarian endometriosis specimens showed high activation of WNT signaling in extraovarian endometriosis, but less in ovarian endometriosis. In extraovarian endometriosis specimens, we detected significantly more nuclear and membranous β-catenin staining, accompanied with increased alteration in target gene expression as compared with endometrium and ovarian endometriosis. Signifying increased canonical WNT

signaling activity especially in extraovarian endometriosis. Membranous β-catenin staining has been shown to be reduced along the transformation from normal endometrium to hyperplasia and cancer [714], and the degradation of membranous β-catenin has been associated with cancer cell metastasis and invasion with various cancer types [715–717], suggesting that β-catenin might have a dual role in endometriosis promoting proliferation, but on the hand controlling the invasion and metastasis processes in endometriosis. Numerous studies have shown the role of SFRP2 either as a WNT signaling agonist or antagonist depending on the tissue and the physiological context [718–726]. Using primary cultured extraovarian endometriosis, in study **II** we showed that SFRP2 knockdown resulted in severely reduced cell proliferation and lower β-catenin protein expression, indicating that SFRP2 expression stimulates the canonical WNT signaling pathway and lesion growth upstream of β-catenin in extraovarian endometriosis. Interestingly, we could not detect gene expression changes in the WNT pathway components between the endometrium of control women and endometriosis patients. Thus, our data suggests non-endometrial origin of the changes in WNT pathway components in endometriosis. Most likely, these changes are gained only after the endometrial tissue has escaped to ectopic location, or they reflect the differential origin of the tissues, as suggested by the metaplasia- or stem cell differentiation -based endometriosis pathogenesis model [727,728].

Surgical management of endometriosis frequently leads to symptomatic improvement [148,729] but about 40 to 50% of women who undertake treatment experience inadequate relief or a swift recurrence of their symptoms [730–732]. Several studies have suggested the partial elimination of endometriosis lesions during surgery as a major cause of endometriosis recurrence [142,733–736] with reports of unseen / undetectable endometriosis or some subclinical forms of the disease [737–740], single cell layer or extremely subtle, or endometriosis may not be easily identified. The use of methylene blue staining of the peritoneum in identifying subtle forms or otherwise undetectable endometriosis (Lewis and Lessey Abstract AAGL (American Association of Gynecologic Laparoscopists) /AGES (Australasian Gynaecological Endoscopy & Surgery) Brisbane, Australia 2008) [741] during surgery has increased the perception that endometriotic cells extend far beyond the visible lesions and that improved visualization of the lesions may be helpful in detecting minimal endometriosis [742,743]. Indigo carmine staining was also recently introduced for this purpose [741]. In extraovarian endometriosis, the SFRP2 protein is expressed in both the epithelium and stroma in both the proliferative and secretory phases of the menstrual cycle and specifically in the stromal area surrounding the endometriotic epithelium. Interestingly in study **II**, we also discovered that SFRP2, marked both the active epithelium and stroma, and consequently, the lesion border of endometriosis, while there was less expression in the adjacent areas of rectovaginal

septum, peritoneum, uterosacral ligaments, or ovarian tissue. In addition, a crucial WNT signaling mediator β-catenin demonstrated similar high expression pattern with SFRP2, suggesting that the expressions of these proteins are interconnected. Notably, SFRP2 and β-catenin displayed a secondary, and more distant lesion border not shown with CD10, indicating that WNT signaling is upregulated further outside the tissue surrounding the primary endometriotic epithelium as compared with CD10, currently used as a reference marker [647,744,745].

6.6 Options for future diagnostics

6.6.1 Diagnostic modalities

The most suitable and up to date approach to diagnosing symptomatic endometriosis non-surgically is enhanced based on a combination of factors such as clinical examination and patient consultations to allow for the identification and selection of women suspected with having endometriosis [211]. Although effortlessly implemented, it is regarded commonly as less accurate than surgical diagnosis. Ballard *et al*. reported increase in the probability of endometriosis the more the number of symptoms present, from an odds ratio of 5.0 with 1 symptom to 84.7 with 7 or more [41]. Due to the invasiveness and the prohibitive cost of laparoscopic surgery, noninvasive diagnostics methods in both clinical practice and scientific studies for endometriosis is of great need. There are currently some noninvasive and less invasive tools which can aid the identification of certain types of endometrial lesions in use [746]. For instance, magnetic resonance imaging or transvaginal ultrasounds can be used to identify ovarian endometriomas and deeply infiltrative endometriosis lesions, such as on the rectovaginal septum, bladder, and sigmoid colon [746,747]. Sensitivity and specificity rates for nonovarian endometriosis using transvaginal ultrasound are 78–98% and 90–100%, respectively [746,748].

6.6.2 Imaging

While diagnostic imaging can be helpful in the diagnosis of endometriosis, it is not without disadvantages and shortcomings. Regarding the best imaging modality, the MRI enables the detection of extremely small lesions and can recognize the hemorrhagic signal of endometriotic lesions due to its extremely high spatial resolution. It is no longer adequate to operate on severe endometriosis without exploring the uterus to exclude the existence of uterine adenomyosis with MRI [749]. Furthermore, it performs better in detecting the limits between muscles and abdominal subcutaneous tissues than the CT scan [750]. MRI has also been demonstrated to precisely detect in more than 90% of cases rectovaginal disease and

obliteration when ultrasonographic gel was inserted in the vagina and rectum [751]. The MRI has a high specificity for identifying endometriomas, categorized by high signal intensity on T1-WI and low signal intensity on T2-WI. Correlation of the imaging features of endometriotic lesion with laparoscopic appearance may help improve individual proficiency in the radiologic diagnosis of endometriosis [752].

MRI diagnostics still requires a dedicated radiologist for high quality diagnosis. And as there are advancements in technologies, clinical symptoms combined with distinctive imaging features and AI in suitable patient groups may facilitate the use of minimally invasive and or noninvasive diagnoses. With AI becoming more pertinent in radiological diagnostics.

6.6.3 Biomarkers

There have been a plethora of evaluations evaluating the specificity and sensitivity of every conceivable invasive and noninvasive diagnostic biochemical marker for diagnosis and or screening for endometriosis. More investment is required in this area to be productive, and it is considered necessary for biomarkers the testing be conducted on populations that mirror the diversity of individuals with the disease. Endometrial and menstrual effluent biomarkers as well as blood based biomarkers under investigation consist of inflammatory markers, growth factors, hormonal markers, tumor markers, and regulators of gene expression (microRNAs) [269,746,753]. However, none have been confirmed in significantly large heterogeneous samples nor have been proven to have sufficient sensitivity and specificity to be used in clinical settings outside of research [746]. Given the heterogeneous nature of endometriosis as well as the various pathways involved in the etiology of the disease, there might not necessarily be a one universal biomarker approach to precisely diagnose all type of endometriosis. A mixture of several biomarkers may be required in conjunction with symptomology to help diagnose the disease or characterize the different subtypes of endometriosis, which could potentially open possibilities for more individualized treatment options. Nevertheless, a large, diverse, and highly phenotypic patient population, with comprehensive prospective data collection on the severity and characteristics of pelvic symptoms (e.g., dysmenorrhea, dyspareunia, non-menstrual pain, infertility), associated complications (e.g., autoimmune disease and other pain conditions), appearance, location, and extent of lesions will be required [746]. The Endometriosis Phenome and Biobanking Harmonization Project established by the World Endometriosis Research Foundation (WERF) aim to achieve this by standardizing the reporting of pathological processing for endometriosis research, and through facilitation of large-scale international collaborations to improve the understanding of the disease [216,217,596–598]. Existing areas of research include predictive biomarkers for early

diagnosis utilizing a metabolomics approach, [431] specific plasma biomarker obtained during menstruation, [754] dynamic contrast-enhanced imaging studies, [755] identification and validation of novel serum markers for early diagnosis, [405] and more.

6.6.4 Benefits of surgical diagnosis

Among patients with clinical suspicion of endometriosis, in 78% to 84% surgical diagnosis with laparoscopy has been demonstrated to confirm the diagnosis supported by histopathological confirmation of both endometrial glands and stroma in biopsies, [756,757] though substantially lower rates have also been reported [258,260,758]. Surgical diagnosis is considered a great tool for the visualization of the pelvis and may assist in identifying the etiology of the pain in the patients, [759] and in the same process surgical ablation of the disease should occur. However, the effectiveness of surgical diagnosis as it pertains to patient outcome is limited, as all the available studies are retrospective. Moreover, there is still a lack of data on the long term outcomes and very little data on the cost effectiveness and quality of life [759].

6.7 Strengths and limitations of studies

We used advanced open-source object-relational database system in both studies **I** and **III**, as opposed to other database systems. Relational databases have numerous advantages and are in many instances an exceptionally viable choice as they are suitable for heavyweight use cases and can easily handle gigabytes of data, while simultaneously serving as a centralized database that multiple applications or user can interface with directly. Open-source software's were also used in the development of the graphical user interface in both studies **I** and **III**. There are numerous advantages to using open-source software from the flexibility and scalability it provides to strong security and lower costs. Using open-source software allows for easy customization and integration, meaning you can start with an open-source baseline and tweak it to your need. Open-source software offer possibilities for a more adaptable technology and for quicker innovation as well as are more dependable since it typically has thousands of independent programmers testing and fixing bugs of the software. Some limitations to using open-source software include issues with compatibility which means the application is developed in a specific environment using specific programming languages, but it can be deployed for use on various systems. Other limitations with using open-source software in developing applications are in support services as well as liabilities and warranties issues as

open-source licenses usually contain only limited warranty and no liability or infringement indemnity protection.

The Endomet database in study **I**, provides a valuable resource for endometriosis functional genomics research and can be used to address additional questions on mRNA expression profiles in endometriosis. One of its core strengths is that it includes the most extensive collection of endometriosis lesions so far analyzed for genome-wide mRNA expression embedded with an interactive web-based user interface that allows researchers investigate mRNA expression related biological questions without the need for advanced computational skills. However, in its current format it only allows for statistical analysis of mRNA data with a few statistical analysis techniques, which is also one of its drawbacks. Another limitation in database study, is that many of the control women recruited in the study, did not present with any typical endometriosis related symptoms. One strength of the Endomet database is that it allows for expansion for future use. Based on its structure, you can add other dataset to the Endomet database and use the analytics tools available to analyze these data. The system is also designed to allow connections from external systems using API. Furthermore, the analytic engine for data analytics in study **I**, uses R as computational engine and easy plug-in of new analytics functions written in R can be easily integrated.

One limitation in study **II**, is that improved extraovarian endometriosis lesion border detection with SFRP2 and β-catenin staining was performed in a relatively small cohort, while larger studies with different endometriosis subtypes in variable cycle phases and under hormonal medication are required to fully evaluate the validity of the method.

In study **III**, the Random Forest (RF) algorithms used are a class of supervised ensemble algorithms that are typically robust to data limitations, such as small sample size. Comparatively, most other supervised algorithms are more susceptible to overfitting because of the relatively small sample sizes. Furthermore, fitting parameters is considerably easier with RF models because one needs to essentially fit only one parameter, and even the default values for the algorithm produces plausible results. Additionally, different randomization runs of the RF optimization procedure reveal the importance of features, in terms of their selection frequency, giving an additional insight on the robustness of the features. Random forest requires little preprocessing, as input features do not need to be scaled. RF can handle categorical and numerical features, which was an advantage in the present study including categorical questionnaire data.

The limitation with using RF is that it lacks interpretability, although it consists of decision trees, the final ensemble model can be hard to interpret. Moreover, when compared to neural networks, RF cannot learn intrinsic low-level, non-linear interactions. Usually, the computational complexity of training RF models is low,

compared to neural networks, but higher than for other standard methods such as naive Bayes or decision trees. Furthermore, RF algorithm may not be robust enough to handle class imbalance in the training datasets and smaller sample size. As with any supervised ML model, the eventual accuracy and applicability of the RF is highly dependent on the training data used. We validated the high accuracy of the model in an independent cohort. However, one potential limitation of the model development are the heathy controls used. Thus, more data is needed to increase the robustness of the algorithm further.

The predictive model in study **III** warrants further testing in a larger cohort and in a more diverse population. Especially, as many of the control women recruited in study **III**, did not present with any typical endometriosis related symptoms. Therefore, having a more diverse study population would likely improve the assessment of a wider applicability of the model in more heterogeneous populations.

6.8 Practical and clinical implications

The results presented in this study adds value to the already existing research data on endometriosis for both women with endometriosis like symptoms and healthcare professionals. With emphasis on translational research, database systems that can bridge basic and clinical sciences are essential. We developed the Endomet database in study **I** for collecting, and processing, basic and clinical science data on endometriosis. The Endomet database is accessed via a user-friendly web-based application, thus increasing data accessibility. Study **I** provide a platform for researchers to investigate mRNA gene expression profile related questions in endometriosis by characterizing and understanding pathways involved in the development of endometriosis.

In study **II,** the high expression of SFRP2 and β-catenin improved endometriosis lesion border detection, which can have implications for better visualization of endometriosis lesions over CD10 in clinical practice. Moreover, SFRP2 acts as a canonical WNT/β-catenin signaling agonist in endometriosis and positively regulates endometriosis lesion growth, suggesting that the WNT pathway may be an important therapeutic target for endometriosis.

There are no quick and easy solutions to reaching a timely diagnosis for endometriosis which may cause diagnostic delays resulting in endometriosis progressing to a more advanced stage and compromising fertility. Symptoms are typically underrecognized among healthcare providers and women with endometriosis like symptoms. Increasing awareness of the disease is one way to facilitate early diagnosis and appropriate intervention [760–763]. As endometriosis symptoms can already start as early as from adolescence, having a screening tool would be beneficial for early intervention. The predictive model in study **III** was

generated to address the issue of diagnostic delays by identifying women at risk of endometriosis early. We expect this predictive tool will help to prioritize women for surgical intervention in clinical practice, hence, speeding up the diagnosis of endometriosis and improving its early management.

7 Conclusions

This interdisciplinary research work encompasses the fields of clinical medicine, information technology, machine learning, and endometriosis research. It addresses challenges in the diagnosis of endometriosis, with the profound impact it exacts on the lives of women suffering from the disease, including associated pain, infertility, decreased QoL, interference with daily life, relationships, and livelihood. The most crucial step in mitigating these negative complications is early diagnosis of the underlying condition. For many, the journey to a concrete diagnosis is long and fraught with obstacles and misdiagnosis. Some of the fundamental challenges include a gold standard of diagnosis based on an invasive surgical procedure and various symptomatology, contributing to the well-established diagnostic delay from first onset of symptoms to surgical diagnosis and management.

To remedy the associated diagnostic delays requires increased patient education, timely referral to healthcare providers, and developing non-invasive diagnostic methods as well as a shift in how endometriosis is approached as a disease by healthcare providers. By approaching endometriosis as a chronic, inflammatory, and heterogenous diseases and focusing on the symptoms, signs, and clinical findings rather than mainly on the surgical findings and pelvic lesions could lead to quick clinical diagnosis and early intervention. In study **III** using this approach with AI and combining these factors into an algorithm we expect to simplify the diagnosis of endometriosis as well as making the process accessible to more clinicians and patients, culminating in early effective management. Therefore, bridging the disparities and minimizing delays in diagnosis and treatment.

In addition, it is of immense importance to discover new medical treatment options, and actively at an earlier age treat women with medications to avoid repeated surgical procedures and to preserve fertility. To achieve this, novel tools for diagnosis and treatment of endometriosis needs to be discovered. We approached this in study **I** by creating an interactive web-based platform the EndometDB, with the analytics tools needed for exploiting genome-wide expression analysis to determine transcriptomics-based classification of endometriosis, and to evaluate novel insights on hormone actions on endometriosis, as well as to assess biomarkers for differentiation of ovarian endometriosis from ovarian cancer without the need for

advanced computational skills. Comparing the gene expression changes of the disease tissue to that of normal healthy tissue is a strong approach to understanding the fundamental cellular events in the etiology of endometriosis. The EndometDB allows for further characterization and description of pathways involved in endometriosis as there is still a need for a more methodical, and complete analysis of the gene expression patterns across the different types of lesions. As the different forms of the lesion may express different markers/genes differently [261]. Evaluating the different lesion types could help to identify potential variety in the etiology of the different endometriosis lesion types. As one example, using the EndometDB we identified Secreted frizzled-related protein 2 (SFRP2) in study **II** to be highly expressed in endometriosis when compared to the endometrium. The protein was shown to be a novel lesion border marker in histological sections, and as a secretory protein it has a potential to serve also as a serum biomarker. There are various therapeutic strategies currently being developed for diseases associated with abnormal WNT signaling [764,765], which includes SFRP2 as a target [719], these strategies could provide new and effective treatment options for endometriosis.

Acknowledgements

The work described in this thesis was conducted at the Department of Obstetrics and Gynecology, Turku University Hospital and University of Turku and at the Institute of Biomedicine, Research Center for Integrative Physiology and Pharmacology (previously Department of Physiology), University of Turku, Finland under the tutelage of Adjunct professor Antti Perheentupa and Professor Matti Poutanen, during 2013 – 2021.

This work would not have been possible without the support of so many people. I would like to thank Professor Emeritus Juha Mäkinen, Professor Emerita Seija Grénman, former heads of the Department of Obstetrics and Gynecology and the current heads of the Department of Obstetrics and Gynecology, Turku University Hospital and University of Turku, Adjunct Professor Karin Mäkikallio-Anttila, Professor Päivi Polo. The head of Institute of Biomedicine Professor Sari Mäkelä, current and former heads of Research Center for Integrative Physiology and Pharmacology, the former head of the Physiology department Professor Jorma Toppari for providing excellent facilities and creating an environment that promotes good collaboration and ensures quality research.

To my supervisors, Adjunct Professor Antti Perheentupa and Professor Matti Poutanen I am eternally grateful and consider myself extremely lucky to have had you both as supervisors. I could not have asked for better mentors, offering advice and encouragement with a perfect blend of insight and humor. Your insightful feedback has pushed me to sharpen my thinking and brought my work to a different level. I have benefited greatly from your wealth of knowledge; I am extremely grateful that you took me on and for your continued faith in me over the years. Thank you for your continued guidance and endless supply of fascinating projects. Your unassuming approach to research and science is a source of inspiration, one I hope to aspire to throughout my scientific career.

Matti you have thought me the principle of academic life like you have done for many, you have pushed, inspired, and supported me throughout this journey. Antti thank you for taking me under your wings and always carving out time even with your busy schedule, your encouraging words and thoughtful, detailed feedback have been invaluable. Thank you for your patience and cheerful outlook.

Thank you, Professor Emeritus Juha Mäkinen as a member of my supervisory committee, your support and discussions are highly appreciated. I am grateful to Professor Matti Nykter and Adjunct Professor Hanna Savolainen-Peltonen for meticulous review of the thesis manuscript. Your valuable remarks, critical review and discussions helped improve the quality of the thesis.

I would like to acknowledge Pirjo Pakarinen's role in bringing me into the physiology family thank you Pirjo. Taija Heinosalo thank you for showing me the ropes when I first came into the group. Kaisa Huhtinen most of the work of this study started with you before passing the on touch to me, thank you for your input over the years you were always at the ready to help with any lingering questions. Pia Suvitie I am forever grateful for your help with the all the clinical aspects of the disease. Thank you for the stimulating conversations over the years it has been an absolute pleasure collaborating with you. Harry Kujari thank you for all your help with the histological evaluation of samples you were never too busy to fit me into your schedule and always ready to assist and thank you for the positive and fascinating conversations about life.

I am grateful to all co-authors and collaborators: Tuomo Komulainen, Vidal Fey, Prem Adhikari, Kalle Rytkönen, Teemu Daniel Laajala, Lila Kallio, Arfa Mehmood, Elina Kaikkonen, Harri Siitari, Arho Virkki, Tero Aittokallio for all your valuable contributions to this body of work. I would also like to thank you Miikka Asukas for your technical assistance.

This study has been conducted as part of the ENDOMET – study. I warmly acknowledge all the women who enabled the studies described in this thesis by participating in it. I would like to express my sincere gratitude to the amazing team of gynecologists that are part of the ENDOMET Team at the Turku University Hospital, a special thank you to Krisi Joronen, Marianne Hallamaa and Carita Edgren, the research nurses Päivi Smedberg in particular, for everything, from patient recruitment to helping systematically collect samples and the dig up clinical data when necessary.

Thank you Pasi Koskmies, it has been a pleasure collaborating with you on different projects during these years.

I would like to thank all alumni and current members of the HSD team and Manuel Tena-Sempere, Petra Sipilä, Lena Strauss, Niina Saarinen-Aaltonen, Suvi Ruohonen, Fu-Ping Zhang, Marika Karikoski, Guillermo Martinez Nieto, Päivi Järvensivu, Heidi Kemiläinen, Matias Knuuttila, Laura Mairinoja, Arttu Junnila, and Krisztina Kukoricza. I would cherish the memories of our time together always from the meetings to the adventures undertook as a group. Your contributions, feedbacks and knowledge has been invaluable to my growth in science. A special thank you to Janne Hakkarainen and Heli Jokela for your friendship during the years. Janne thank you for welcoming me with open arms when I came to the group you made my

integration much easier. Heli for your positive attitude always. Hanna Heikelä, and Riikka Huhtaniemi best office mates ever thank you for all the fun time in the office. A special thank you to all current and past HSD team research technicians Anu Salminen, Satu Orasniemi and Madeleine Latvala your invaluable input in the body of work is much appreciated. Thank you Anu for guiding me through the technical part in the lab your patience in the early part is much appreciated.

I can't think of a better place or group of people to work with than at the Physiology department. The atmosphere, environment, and the synergy between the different research groups has been great. I want to express my sincere gratitude to all the professors and principal investigators of our great department, Professor Emeritus Ilpo Huhtaniemi, Noora Kotaja, Nafis Rahman, Jukka Kero, Marko Kallio, Harri Niinikoski. It has been a pleasure to work with you all. All previous and present senior scientists, post-docs and PhDs at our department, Helena Virtanen, Adolfo Rivero-Müller, Marcin Chruschiel, Mirja Nurmio, Mari Vähä-Mäkilä, Annika Adamsson, Holger Jäschke, Marion Adam, Juho-Antti Mäkelä, Matti Lahti, Christoffer Löf, Heikki Turunen, Oliver Meikar, Matteo Da Ros, Ida Björkgren, Emmi Rotgers, Jenni Mäki-Jouppila, Hanna Korhonen, Mari Lehti, Mahesh Tambe, Donata Ponikwicka-Tyszko, Ashutosh Trehan, Henriette Undeutsch, Natalia Gruvits, Jaakko Koskenniemi, Milena Doroszko, Sergey Sadov, Valeriy Paramonov, Mehrad Mahmoudian, and Ram Prakash Yadav. I wish to also thank all the doctoral candidates and undergraduate students for their help and pleasant discussions throughout the years: Konrad Patyra, Opeyemi Olotu, Wiwat Rodpraset, Anna Eggert, Matthieu Bourgery, Kamila Pulawska, Lin Ma, Ammar Ahmedani, Margareeta Mäkelä, Titta Kivikoski, Juho Asteljoki, Meeri Jännäri, Lili Niinimäki, Aleksi Hakkarainen, Fatemeh Hajati, and Tiina Lahtela.

I would like to thank all the past and present technical personnel at the department for all you do to make us all efficient, you are all unsung heroes. Thank you Tuula Hämäläinen for running an efficient lab and keeping us all in line, for all the kind words and assistance you have provided over the years. Minna Lindroth and Johanna Järvi for helping take care of orders, Marko Tirri, for your help with technical issues. I appreciate your inputs in making the work life easier for us all. Heidi Liljenbäck, Nina Messner, Mona Niiranen, Heli Niittymäki, Jonna Palmu, Taija Poikkipuoli, Jenni Airaksinen, Paulina Toivonen, Janne Sulku, Elina Louramo and Leena Karlsson without your help and assistance, it would not have been possible to do science. The collaboration and help from the Histocore has been invaluable. Thank you, Erica Nyman and Marja-Riitta Kajaala for all your help with histological analysis.

I want to warmly acknowledge my BIMA mates: Prince Dadson, Kumail Montiani, Elnaz Fazeli, Marjukka Elmeranta, Kalin Lin, Sina Tadayon, Madis

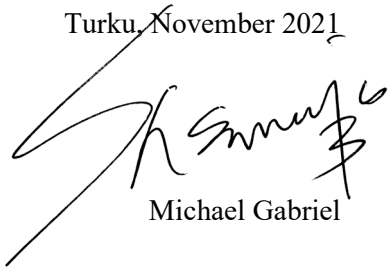
Lohmus, Mojtaba Jafari Tadi, and Sanaz Nazari Farsani thank you all for the memories.

I have been fortunate enough to meet so many amazing people throughout this journey. It is important to strike a balance with life outside of work as such I would like to express special gratitude to Tiina Lehtiniemi, Gabriela Martinez Chacón, Sheyla Cisneros-Montalvo, Hanna Heikelä and Katja Eubler for the great company, brightening my days and cheering on me to get this over the finish line, all those dancing nights, gym, movie nights, karaoke, trips together, encouraging talks, while we might no longer be colleagues at work, we will forever remain friends.

Finally, I want to thank my loved ones. My parents for your endless support throughout the years thank you. You have always stood behind me, and this is no exception. Your words of encouragement and push for tenacity ring in my ears. Your good examples have taught me to work hard for the things that I aspire to achieve. Mom, thank you for fielding a ridiculous amount of phone calls, for calming me down when I am stressed. To all my siblings Sam, Tope, Elizabeth, Sarah, Solomon, and Daniel thank you all for always being there encouraging me. To my extended family Uncle Femi, Aunty Funmi, Aunty Titi, Madina Daff, Funke, Debbie, Bolu, Tito, Sarah-Maya, Moses, and Ami-Ruth, thank you all for your unconditional love and support. Галина Васильевна, спасибо за вашу огромную щедрость и за то, что принимаете меня как родного. Наталья Валерьевна ты была постоянным источником поддержки и ободрения во время жизненных испытаний. Я искренне благодарен за то, что ты есть в моей жизни. Спасибо за наших прекрасных мальчиков. To Samuel and David thank you for bringing so much joy and love to my everyday life.

This work was financially supported by the Academy of Finland; Finnish Funding Agency for Technology and Innovation (Business Finland); The Hospital District of Southwest Finland; Sigrid Jusélius Foundation; The Turku University Hospital, University of Turku, Turku Finland; Forendo Pharma; Clinical Research (EVO) fund of the Turku University Hospital; Doctoral Programme in Clinical Research (DPCR); and Turku University Foundation.

Turku, November 2021



Michael Gabriel

References

1. Giudice, L. C. Endometriosis. *N. Engl. J. Med.* **362**, 2389–2398 (2010).
2. Kennedy, S. *et al.* ESHRE guideline for the diagnosis and treatment of endometriosis. *Hum. Reprod.* **20**, 2698–2704 (2005).
3. Johnson, N. P. & Hummelshoj, L. Consensus on current management of endometriosis. *Hum. Reprod.* **28**, 1552–1568 (2013).
4. Siedentopf, F., Tariverdian, N., Rücke, M., Kentenich, H. & Arck, P. C. Immune status, psychosocial distress and reduced quality of life in infertile patients with endometriosis. *Am. J. Reprod. Immunol.* (2008) doi:10.1111/j.1600-0897.2008.00644.x.
5. De Graaff, A. A. *et al.* The significant effect of endometriosis on physical, mental and social wellbeing: Results from an international cross-sectional survey. *Hum. Reprod.* **28**, 2677–2685 (2013).
6. Simoens, S. *et al.* The burden of endometriosis: Costs and quality of life of women with endometriosis and treated in referral centres. *Hum. Reprod.* **27**, 1292–1299 (2012).
7. Nnoaham, K. E. *et al.* Impact of endometriosis on quality of life and work productivity: A multicenter study across ten countries. *Fertil. Steril.* **96**, (2011).
8. Guo, S. W. Epigenetics of endometriosis. *Mol. Hum. Reprod.* **15**, 587–607 (2009).
9. Johnson, N. P. *et al.* World Endometriosis Society consensus on the classification of endometriosis. *Hum. Reprod.* **32**, 315–324 (2017).
10. Canis, M. *et al.* Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil. Steril.* **67**, 817–821 (1997).
11. Medicine, A. S. for R. *FERTILITY AND STERILITY® Special contribution Revised American Society for Reproductive Medicine classification of endometriosis: 1996 American Society for Reproductive Medicine*t*. *Fertility and Sterility* vol. 67 (1997).
12. Classification of endometriosis. *Fertil. Steril.* **32**, 633–634 (1979).
13. Andrews, W. C., Buttram, V. C. & Behrman, S. J. Revised American fertility society classification of endometriosis: 1985. *Fertil. Steril.* **44**, 7–8 (1985).
14. Nisolle, M. & Donnez, J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. *Fertil. Steril.* **68**, 585–596 (1997).
15. Vercellini, P., Viganò, P., Somigliana, E. & Fedele, L. Endometriosis: Pathogenesis and treatment. *Nat. Rev. Endocrinol.* **10**, 261–275 (2014).
16. De Mauro, A., Greco, M. & Grimaldi, M. A formal definition of Big Data based on its essential features. *Library Review* vol. 65 122–135 (2016).
17. Hashem, I. A. T. *et al.* The rise of 'big data' on cloud computing: Review and open research issues. *Information Systems* vol. 47 98–115 (2015).
18. Vogel, C. *et al.* A Delphi study to build consensus on the definition and use of big data in obesity research. *Int. J. Obes.* **43**, 2573–2586 (2019).
19. Obermeyer, Z. & Emanuel, E. J. Predicting the future-big data, machine learning, and clinical medicine. *New England Journal of Medicine* vol. 375 1216–1219 (2016).
20. Murdoch, T. B. & Detsky, A. S. The inevitable application of big data to health care. *JAMA - Journal of the American Medical Association* vol. 309 1351–1352 (2013).

21. Ballard, L. A. *Comprehensive Gynecology*. *Cleveland Clinic Journal of Medicine* vol. 55 (Elsevier, 1988).
22. Sampson, J. A. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am. J. Obstet. Gynecol.* **14**, 422–469 (1927).
23. Sonavane, S. K., Kantawala, K. P. & Menias, C. O. Beyond the Boundaries-Endometriosis: Typical and Atypical Locations. *Current Problems in Diagnostic Radiology* vol. 40 219–232 (2011).
24. Thibodeau, L. L., Prioleau, G. R., Manuelidis, E. E., Merino, M. J. & Heafner, M. D. Cerebral endometriosis. Case report. *J. Neurosurg.* **66**, 609–610 (1987).
25. Patel, V. C., Samuels, H., Abeles, E. & Hirjibehedin, P. F. Endometriosis at the knee. A case report. *Clin. Orthop. Relat. Res.* **No. 171**, 140–144 (1982).
26. Machairiotis, N. *et al.* Extrapelvic endometriosis: A rare entity or an under diagnosed condition? *Diagnostic Pathology* vol. 8 194 (2013).
27. Faske, E. J., MacK, L. M. & Ozcan, T. Incidental finding of decidualized vesical endometriosis in an asymptomatic obstetrical patient. *J. Ultrasound Med.* **31**, 809–811 (2012).
28. Rivkine, E. *et al.* Hepatic endometrioma: A case report and review of the literature: Report of a case. *Surg. Today* **43**, 1188–1193 (2013).
29. Liu, K., Zhang, W., Liu, S., Dong, B. & Liu, Y. Hepatic endometriosis: A rare case and review of the literature. *Eur. J. Med. Res.* **20**, (2015).
30. Markham, S. M., Carpenter, S. E. & Rock, J. A. Extrapelvic endometriosis. *Obstet. Gynecol. Clin. North Am.* **16**, 193–219 (1989).
31. Agarwal, N. & Subramanian, A. Endometriosis - Morphology, clinical presentations and molecular pathology. *J. Lab. Physicians* **2**, 001–009 (2010).
32. Bulun, S. E. Endometriosis. *New England Journal of Medicine* vol. 360 268–279 (2009).
33. Bulun, S. E. *et al.* Role of estrogen receptor-β in endometriosis. *Semin. Reprod. Med.* **30**, 39–45 (2012).
34. Missmer, S. A. *et al.* Incidence of Laparoscopically Confirmed Endometriosis by Demographic, Anthropometric, and Lifestyle Factors. *Am. J. Epidemiol. Hopkins Bloom. Sch. Public Heal. All rights Reserv.* **160**, 784–796 (2004).
35. Hediger, M. L., Hartnett, H. J. & Louis, G. M. B. Association of endometriosis with body size and figure. *Fertil. Steril.* **84**, 1366–1374 (2005).
36. Matalliotakis, I. M. *et al.* Familial aggregation of endometriosis in the Yale Series. *Arch. Gynecol. Obstet.* **278**, 507–511 (2008).
37. Malinak, L. R., Buttram, V. C., Elias, S. & Simpson, J. L. Heritable aspects of endometriosis. II. Clinical characteristics of familial endometriosis. *Am. J. Obstet. Gynecol.* **137**, 332–337 (1980).
38. Ahn, S. H., Singh, V. & Tayade, C. *Biomarkers in endometriosis: challenges and opportunities*. *Fertility and Sterility* vol. 107 523–532 (Elsevier Inc., 2017).
39. Jansen, F. W., Kapiteyn, K., Trimbos-Kemper, T., Hermans, J. & Trimbos, J. B. Complications of laparoscopy: A prospective multicentre observational study. *BJOG An Int. J. Obstet. Gynaecol.* **104**, 595–600 (1997).
40. Chapron, C. *et al. Surgical complications of diagnostic and operative gynaecological laparoscopy: A series of 29,966 cases*. *Human Reproduction* vol. 13 (1998).
41. Ballard, K. D. K., Seaman, H. E. H. E., de Vries, C. S. C. & Wright, J. T. Can symptomatology help in the diagnosis of endometriosis? Findings from a national case-control study - Part 1. *BJOG An Int. J. Obstet. Gynaecol.* **115**, 1382–1391 (2008).
42. Eskenazi, B. & Warner, M. L. Epidemiology of endometriosis. *Obstet. Gynecol. Clin. North Am.* **24**, 235–258 (1997).
43. Rogers, P. A. W. *et al.* Priorities for endometriosis research: Recommendations from an international consensus workshop. *Reprod. Sci.* **16**, 335–346 (2009).
44. David Adamson, G., Kennedy, S. & Hummelshoj, L. Creating solutions in endometriosis: Global collaboration through the World Endometriosis Research Foundation. *J. Endometr.* **2**, 3–6 (2010).

45. Hickey, M., Ballard, K. & Farquhar, C. Endometriosis. *BMJ (Online)* vol. 348 (2014).
46. Hadfield, R., Mardon, H., Barlow, D. & Kennedy, S. Delay in the diagnosis of endometriosis: a survey of women from the USA and the UK. *Hum. Reprod.* **11**, 878–880 (1996).
47. Husby, G. K., Haugen, R. S. & Moen, M. H. Diagnostic delay in women with pain and endometriosis. *Acta Obstet. Gynecol. Scand.* **82**, 649–653 (2003).
48. Ballard, K., Lowton, K. & Wright, J. What's the delay? A qualitative study of women's experiences of reaching a diagnosis of endometriosis. *Fertil. Steril.* **86**, 1296–1301 (2006).
49. Hudelist, G. *et al.* Diagnostic delay for endometriosis in Austria and Germany: causes and possible consequences. *Hum. Reprod.* **27**, 3412–3416 (2012).
50. Nnoaham, K. E. *et al.* Impact of endometriosis on quality of life and work productivity: A multicenter study across ten countries. *Fertil. Steril.* **96**, 366-373.e8 (2011).
51. Morassutto, C., Monasta, L., Ricci, G., Barbone, F. & Ronfani, L. Incidence and estimated prevalence of endometriosis and adenomyosis in Northeast Italy: A data linkage study. *PLoS One* **11**, e0154227 (2016).
52. Agarwal, S. K. *et al.* Clinical diagnosis of endometriosis: a call to action. *Am. J. Obstet. Gynecol.* **220**, 354.e1-354.e12 (2019).
53. Gao, X. *et al.* A figure is presented Economic burden of endometriosis. *Fertility and Sterility* vol. 86 1561–1572 (2006).
54. Simoens, S., Hummelshoj, L. & D'Hooghe, T. Endometriosis: Cost estimates and methodological perspective. *Human Reproduction Update* vol. 13 395–404 (2007).
55. Lemos, N. A. *et al.* Decreased anti-Müllerian hormone and altered ovarian follicular cohort in infertile patients with mild/minimal endometriosis. *Fertil. Steril.* **89**, 1064–1068 (2008).
56. Kitajima, M. *et al.* Endometriomas as a possible cause of reduced ovarian reserve in women with endometriosis. *Fertil. Steril.* **96**, 685–691 (2011).
57. Maneschi, F., Marasá, L., Incandela, S., Mazzarese, M. & Zupi, E. Ovarian cortex surrounding benign neoplasms: A histologic study. *Am. J. Obstet. Gynecol.* **169**, 388–393 (1993).
58. The Effect of Surgery for Endometriomas on Fertility: Scientific Impact Paper No. 55. *BJOG An Int. J. Obstet. Gynaecol.* **125**, e19–e28 (2018).
59. Agarwal, A., Aponte-Mellado, A., Premkumar, B. J., Shaman, A. & Gupta, S. The effects of oxidative stress on female reproduction: A review. *Reproductive Biology and Endocrinology* vol. 10 49 (2012).
60. Gibson, D. A., Simitsidellis, I., Collins, F. & Saunders, P. T. K. Endometrial intracrinology: Oestrogens, androgens and endometrial disorders. *Int. J. Mol. Sci.* **19**, (2018).
61. Carpinello, O. J., Sundheimer, L. W., Alford, C. E., Taylor, R. N. & DeCherney, A. H. *Endometriosis*. *Endotext* (MDText.com, Inc., 2000).
62. Newman, T. A. *et al.* Expression of neuronal markers in the endometrium of women with and those without endometriosis. *Hum. Reprod.* **28**, 2502–2510 (2013).
63. Chatman, D. L. Pelvic peritoneal defects and endometriosis: Allen-Masters syndrome revisited. *Fertil. Steril.* **36**, 751–756 (1981).
64. Cornillie, F. J., Oosterlynck, D., Lauweryns, J. M. & Koninckx, P. R. Deeply infiltrating pelvic endometriosis: histology and clinical significance. *Fertil. Steril.* **53**, 978–983 (1990).
65. Chapron, C. *et al.* Anatomical distribution of deeply infiltrating endometriosis: Surgical implications and proposition for a classification. *Hum. Reprod.* **18**, 157–161 (2003).
66. Chapron, C. & Dubuisson, J. B. *Laparoscopic treatment of deep endometriosis located on the uterosacral ligaments*. *Human Reproduction* vol. 11 https://academic.oup.com/humrep/article/11/4/868/624875 (1996).
67. Chapron, C. *et al.* Deep infiltrating endometriosis: Relation between severity of dysmenorrhoea and extent of disease. *Hum. Reprod.* **18**, 760–766 (2003).
68. Martin, D. C. & Batt, R. E. Retrocervical, rectovaginal pouch, and rectovaginal septum endometriosis. *Journal of the American Association of Gynecologic Laparoscopists* vol. 8 12–17 (2001).

69. Buchweitz, O., Poel, T., Diedrich, K. & Malik, E. The diagnostic dilemma of minimal and mild endometriosis under routine conditions. *J. Am. Assoc. Gynecol. Laparosc.* **10**, 85–89 (2003).
70. Tissot, M. *et al.* Clinical presentation of endometriosis identified at interval laparoscopic tubal sterilization: Prospective series of 465 cases. *J. Gynecol. Obstet. Hum. Reprod.* **46**, 647–650 (2017).
71. Moen, M. H. & Stokstad, T. A long-term follow-up study of women with asymptomatic endometriosis diagnosed incidentally at sterilization. in *Fertility and Sterility* vol. 78 773–776 (Fertil Steril, 2002).
72. Milingos, S. *et al.* Laparoscopic management of patients with endometriosis and chronic pelvic pain. *Ann. N. Y. Acad. Sci.* **997**, 269–273 (2003).
73. Fuldeore, M. J. & Soliman, A. M. Prevalence and Symptomatic Burden of Diagnosed Endometriosis in the United States: National Estimates from a Cross-Sectional Survey of 59,411 Women. *Gynecol. Obstet. Invest.* **82**, 453–461 (2017).
74. Schliep, K. C. *et al.* Pain typology and incident endometriosis. *Hum. Reprod.* **30**, 2427–2438 (2015).
75. Peterson, C. M. *et al.* Risk factors associated with endometriosis: Importance of study population for characterizing disease in the ENDO Study. *Am. J. Obstet. Gynecol.* **208**, 451.e1-451.e11 (2013).
76. Laux-Biehlmann, A., D'hooghe, T. & Zollner, T. M. Menstruation pulls the trigger for inflammation and pain in endometriosis. *Trends in Pharmacological Sciences* vol. 36 270–276 (2015).
77. Ilangavan, K. & Kalu, E. High prevalence of endometriosis in infertile women with normal ovulation and normospermic partners. *Fertility and Sterility* vol. 93 e10; author reply e12 (2010).
78. Meuleman, C. *et al.* High prevalence of endometriosis in infertile women with normal ovulation and normospermic partners. *Fertil. Steril.* **92**, 68–74 (2009).
79. Cranney, R., Condous, G. & Reid, S. An update on the diagnosis, surgical management, and fertility outcomes for women with endometrioma. *Acta Obstetricia et Gynecologica Scandinavica* vol. 96 633–643 (2017).
80. Evans, M. B. & Decherney, A. H. Fertility and Endometriosis. *Clin. Obstet. Gynecol.* **60**, 497–502 (2017).
81. Macer, M. L. & Taylor, H. S. Endometriosis and Infertility. A Review of the Pathogenesis and Treatment of Endometriosis-associated Infertility. *Obstetrics and Gynecology Clinics of North America* vol. 39 535–549 (2012).
82. Hirsch, M. *et al.* Diagnosis and management of endometriosis: a systematic review of international and national guidelines. *BJOG An Int. J. Obstet. Gynaecol.* **125**, 556–564 (2018).
83. Practice bulletin no. 114: Management of endometriosis. *Obstet. Gynecol.* **116**, 223–236 (2010).
84. Practice Committee of the American Society for Reproductive Medicine. Treatment of pelvic pain associated with endometriosis: A committee opinion. *Fertil. Steril.* **101**, 927–935 (2014).
85. Schleedoorn, M. J. *et al.* Selection of key recommendations for the management of women with endometriosis by an international panel of patients and professionals. *Hum. Reprod.* **31**, 1208–1218 (2016).
86. Brown, J. & Farquhar, C. Endometriosis: An overview of Cochrane Reviews. *Cochrane Database of Systematic Reviews* vol. 2014 (2014).
87. Funk, C. D. Prostaglandins and leukotrienes: Advances in eicosanoid biology. *Science* vol. 294 1871–1875 (2001).
88. Banu, S. K., Lee, J., Speights, V. O., Starzinski-Powitz, A. & Arosh, J. A. Cyclooxygenase-2 regulates survival, migration, and invasion of human endometriotic cells through multiple mechanisms. *Endocrinology* **149**, 1180–1189 (2008).
89. Fan, H. & Fang, X. L. Expression of cylooxygenase-2 in endometriosis. *J. Cent. South Univ. (Medical Sci.* **30**, 92–95 (2005).

90. Carli, C., Metz, C. N., Al-Abed, Y., Naccache, P. H. & Akoum, A. Up-regulation of cyclooxygenase-2 expression and prostaglandin E 2 production in human endometriotic cells by macrophage migration inhibitory factor: Involvement of novel kinase signaling pathways. *Endocrinology* **150**, 3128–3137 (2009).
91. Ota, H., Igarashi, S., Sasaki, M. & Tanaka, T. Distribution of cyclooxygenase-2 in eutopic and ectopic endometrium in endometriosis and adenomyosis. *Hum. Reprod.* **16**, 561–566 (2001).
92. Willman, E. A., Collins, W. P. & Clayton, S. G. Studies in the Involvement of Prostaglandins in Uterine Symptomatology and Pathology. *BJOG An Int. J. Obstet. Gynaecol.* **83**, 337–341 (1976).
93. Lundström, V., Gréen, K. & Svanborg, K. Endogenous Prostaglandins in Dysmenorrhea and the Effect of Prostaglandin Synthetase Inhibitors (Pgsi) on Uterine Contractility. *Acta Obstet. Gynecol. Scand.* **58**, 51–56 (1979).
94. STRÖMBERG, P., ÅKERLUND, M., FORSLING, M. L. & KINDAHL, H. Involvement of prostaglandins in vasopressin stimulation of the human uterus. *BJOG An Int. J. Obstet. Gynaecol.* **90**, 332–337 (1983).
95. Muzii, L. *et al.* Continuous versus cyclic oral contraceptives after laparoscopic excision of ovarian endometriomas: A systematic review and metaanalysis. *American Journal of Obstetrics and Gynecology* vol. 214 203–211 (2016).
96. Zorbas, K. A., Economopoulos, K. P. & Vlahos, N. F. Continuous versus cyclic oral contraceptives for the treatment of endometriosis: a systematic review. *Archives of Gynecology and Obstetrics* vol. 292 37–43 (2015).
97. Whitehead, M. I. *et al.* Effects of various types and dosages of progestogens on the postmenopausal endometrium. *J. Reprod. Med. Obstet. Gynecol.* **27**, 539–548 (1982).
98. Bruner, K. L., Eisenberg, E., Gorstein, F. & Osteen, K. G. Progesterone and transforming growth factor-β coordinately regulate suppression of endometrial matrix metalloproteinases in a model of experimental endometriosis. *Steroids* **64**, 648–653 (1999).
99. Moghissi, K. S. & Boyce, C. R. Management of endometriosis with oral medroxyprogesterone acetate. *Obstet. Gynecol.* **47**, 265–267 (1976).
100. Luciano, A. A., Nuran Turksoy, R. N. & Carleo, J. Evaluation of oral medroxyprogesterone acetate in the treatment of endometriosis. *Obstet. Gynecol.* **72**, 323–327 (1988).
101. Vercellini, P. *et al.* Treatment of symptomatic rectovaginal endometriosis with an estrogen-progestogen combination versus low-dose norethindrone acetate. *Fertil. Steril.* **84**, 1375–1387 (2005).
102. Vercellini, P. *et al.* Endometriosis: Current therapies and new pharmacological developments. *Drugs* vol. 69 649–675 (2009).
103. Treatment of pelvic pain associated with endometriosis. *Fertil. Steril.* **90**, S260–S269 (2008).
104. European Society of Human Reproduction and Embryology (ESHRE). Endometriosis guideline. https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Endometriosis-guideline (2013).
105. Leyland, N. *et al.* Endometriosis: Diagnosis and Management. *J. Obstet. Gynaecol. Canada* **32**, S1–S3 (2010).
106. Schindler, A. E. Dienogest in long-term treatment of endometriosis. *International Journal of Women's Health* vol. 3 175–184 (2011).
107. Köhler, G., Faustmann, T. A., Gerlinger, C., Seitz, C. & Mueck, A. O. A dose-ranging study to determine the efficacy and safety of 1, 2, and 4 mg of dienogest daily for endometriosis. *Int. J. Gynecol. Obstet.* **108**, 21–25 (2010).
108. Gezer, A. & Oral, E. Progestin therapy in endometriosis. *Women's Heal.* **11**, 643–652 (2015).
109. Vercellini, P. *et al.* A levonorgestrel-releasing intrauterine system for the treatment of dysmenorrhea associated with endometriosis: A pilot study. *Fertil. Steril.* **72**, 505–508 (1999).
110. Petta, C. A. *et al.* Randomized clinical trial of a levonorgestrel-releasing intrauterine system and a depot GnRH analogue for the treatment of chronic pelvic pain in women with endometriosis. *Hum. Reprod.* **20**, 1993–1998 (2005).

111. Vercellini, P. *et al.* Comparison of a levonorgestrel-releasing intrauterine device versus expectant management after conservative surgery for symptomatic endometriosis: A pilot study. *Fertil. Steril.* **80**, 305–309 (2003).
112. Brown, J., Pan, A. & Hart, R. J. Gonadotrophin-releasing hormone analogues for pain associated with endometriosis. *Cochrane Database Syst. Rev.* (2010) doi:10.1002/14651858.CD008475.pub2.
113. Prentice, A., Deary, A., Goldbeck-Wood, S., Farquhar, C. & Smith, S. Gonadotrophin-releasing hormone analogues for pain associated with endometriosis. *Cochrane Database Syst. Rev.* (1999) doi:10.1002/14651858.cd000346.pub2.
114. Winkel, C. A. & Scialli, A. R. Medical and surgical therapies for pain associated with endometriosis. *Journal of Women's Health and Gender-Based Medicine* vol. 10 137–162 (2001).
115. Bulun, S. E., Zeitoun, K. M., Takayama, K. & Sasano, H. Molecular basis for treating endometriosis with aromatase inhibitors. *Human Reproduction Update* vol. 6 413–418 (2000).
116. Agarwal, S. K. & Foster, W. G. Reduction in Endometrioma Size with Three Months of Aromatase Inhibition and Progestin Add-Back. *Biomed Res. Int.* **2015**, 878517 (2015).
117. Abu Hashim, H. Potential role of aromatase inhibitors in the treatment of endometriosis. *International Journal of Women's Health* vol. 6 671–680 (2014).
118. Nothnick, W. B. The emerging use of aromatase inhibitors for endometriosis treatment. *Reproductive Biology and Endocrinology* vol. 9 87 (2011).
119. Pavone, M. E. & Bulun, S. E. Aromatase inhibitors for the treatment of endometriosis. *Fertility and Sterility* vol. 98 1370–1379 (2012).
120. Rafique, S. & Decherney, A. H. Medical Management of Endometriosis. *Clin. Obstet. Gynecol.* **60**, 485–496 (2017).
121. Taylor, H. S. *et al.* Novel therapies targeting endometriosis. *Reproductive Sciences* vol. 18 814–823 (2011).
122. Pinkerton, J. V. & Thomas, S. Use of SERMs for treatment in postmenopausal women. *Journal of Steroid Biochemistry and Molecular Biology* vol. 142 142–154 (2014).
123. Chabbert-Buffet, N., Pintiaux, A. & Bouchard, P. The immninent dawn of SPRMs in obstetrics and gynecology. *Molecular and Cellular Endocrinology* vol. 358 232–243 (2012).
124. Huniadi, C. A., Pop, O. L., Antal, T. A. & Stamatian, F. The effects of ulipristal on Bax/Bcl-2, cytochrome C, Ki-67 and cyclooxygenase-2 expression in a rat model with surgically induced endometriosis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **169**, 360–365 (2013).
125. Mozzanega, B., Gizzo, S., Di Gangi, S., Cosmi, E. & Nardelli, G. B. Ulipristal acetate: Critical review about endometrial and ovulatory effects in emergency contraception. *Reproductive Sciences* vol. 21 678–685 (2014).
126. Zito, G. *et al.* Medical treatments for endometriosis-associated pelvic pain. *Biomed Res. Int.* **2014**, (2014).
127. Bouchard, P., Chabbert-Buffet, N. & Fauser, B. C. J. M. Selective progesterone receptor modulators in reproductive medicine: Pharmacology, clinical efficacy and safety. *Fertil. Steril.* **96**, 1175–1189 (2011).
128. Kulak, J., Fischer, C., Komm, B. & Taylor, H. S. Treatment with bazedoxifene, a selective estrogen receptor modulator, causes regression of endometriosis in a mouse model. *Endocrinology* **152**, 3226–3232 (2011).
129. Bedaiwy, M. A., Alfaraj, S., Yong, P. & Casper, R. New developments in the medical treatment of endometriosis. *Fertility and Sterility* vol. 107 555–565 (2017).
130. Altintas, D., Kokcu, A., Kandemir, B., Tosun, M. & Cetinkaya, M. B. Comparison of the effects of raloxifene and anastrozole on experimental endometriosis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **150**, 84–87 (2010).
131. Shang, Y. Molecular mechanisms of oestrogen and SERMs in endometrial carcinogenesis. *Nature Reviews Cancer* vol. 6 360–368 (2006).

132. Carr, B. *et al.* Elagolix, an oral GnRH antagonist, versus subcutaneous depot medroxyprogesterone acetate for the treatment of endometriosis: Effects on bone mineral density. *Reprod. Sci.* **21**, 1341–1351 (2014).
133. Pluchino, N., Freschi, L., Wenger, J. M. & Streuli, I. Innovations in classical hormonal targets for endometriosis. *Expert Review of Clinical Pharmacology* vol. 9 317–327 (2016).
134. Naqvi, H. *et al.* Treatment with bazedoxifene and conjugated estrogens results in regression of endometriosis in a murine model. *Biol. Reprod.* **90**, (2014).
135. Goyeneche, A. A. & Telleria, C. M. Antiprogestins in gynecological diseases. *Reproduction* vol. 149 R15–R33 (2015).
136. Chwalisz, K., Garg, R., Brenner, R. M., Schubert, G. & Elger, W. Selective progesterone receptor modulators (SPRMs): A novel therapeutic concept in endometriosis. in *Annals of the New York Academy of Sciences* vol. 955 373–388 (New York Academy of Sciences, 2002).
137. Chwalisz, K., Brenner, R. M., Fuhrmann, U. U., Hess-Stumpp, H. & Elger, W. Antiproliferative effects of progesterone antagonists and progesterone receptor modulators on the endometrium. in *Steroids* vol. 65 741–751 (2000).
138. Wells, B. G., DiPiro, J. T., Schwinghammer, T. L. & DiPiro, C. V. *Pharmacotherapy Handbook: Ninth Edition*. (McGraw-Hill Education - Europe, 2014).
139. Vercellini, P. *et al.* Surgery for deep endometriosis: A pathogenesis-oriented approach. *Gynecol. Obstet. Invest.* **68**, 88–103 (2009).
140. Crosignani, P. G. *et al.* Laparoscopy versus laparotomy in conservative surgical treatment for severe endometriosis. *Fertil. Steril.* **66**, 706–711 (1996).
141. Duffy, J. M. N. *et al.* Laparoscopic surgery for endometriosis. *Cochrane Database of Systematic Reviews* vol. 2014 (2014).
142. Guo, S. W. Recurrence of endometriosis and its control. *Hum. Reprod. Update* **15**, 441–461 (2009).
143. Olive, D. L. & Pritts, E. Treatment of endometriosis. *N. Engl. J. Med.* **345**, 266–275 (2001).
144. Vercellini, P. *et al.* The effect of surgery for symptomatic endometriosis: The other side of the story. *Hum. Reprod. Update* **15**, 177–188 (2009).
145. Clarke, J. Laparoscopic surgery for pelvic pain associated with endometriosis. *Journal of Pain Management* vol. 3 337–339 (2010).
146. Jacobson, T. Z., Duffy, J. M. N., Barlow, D. H., Koninckx, P. R. & Garry, R. Laparoscopic surgery for pelvic pain associated with endometriosis. *Cochrane Database of Systematic Reviews* vol. 2014 (2014).
147. Vercellini, P. *et al.* Repetitive surgery for recurrent symptomatic endometriosis: What to do? *European Journal of Obstetrics and Gynecology and Reproductive Biology* vol. 146 15–21 (2009).
148. Sutton, C. J. G., Ewen, S. P., Whitelaw, N. & Haines, P. Prospective, randomized, double-blind, controlled trial of laser laparoscopy in the treatment of pelvic pain associated with minimal, mild, and moderate endometriosis. *ACOG Curr. J. Rev.* **62**, 696–700 (1995).
149. Abbott, J. *et al.* Laparoscopic excision of endometriosis: A randomized, placebo-controlled trial. *Fertil. Steril.* **82**, 878–884 (2004).
150. Pundir, J. *et al.* Laparoscopic Excision Versus Ablation for Endometriosis-associated Pain: An Updated Systematic Review and Meta-analysis. *Journal of Minimally Invasive Gynecology* vol. 24 747–756 (2017).
151. Somigliana, E. *et al.* Surgical excision of endometriomas and ovarian reserve: A systematic review on serum antimüllerian hormone level modifications. *Fertil. Steril.* **98**, 1531–1538 (2012).
152. Sourial, S., Tempest, N. & Hapangama, D. K. Theories on the Pathogenesis of Endometriosis. *Int. J. Reprod. Med.* **2014**, 1–9 (2014).
153. Nisolle, M. & Donnez, J. Reprint of: Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. *Fertil. Steril.* **112**, e125–e136 (2019).

154. Moen, M. H. & Magnus, P. The familial risk of endometriosis. *Acta Obstet. Gynecol. Scand.* **72**, 560–564 (1993).
155. Giudice, L. C. & Kao, L. C. Endometriosis. in *Lancet* vol. 364 1789–1799 (Elsevier, 2004).
156. Dun, E. C., Taylor, R. N. & Wieser, F. Advances in the genetics of endometriosis. *Genome Medicine* vol. 2 75 (2010).
157. Deiana, D. *et al.* Genetics of endometriosis: a comprehensive review. *Gynecol. Endocrinol.* **35**, 553–558 (2019).
158. Rier, S. Endometriosis in Rhesus Monkeys (Macaca mulatta) Following Chronic Exposure to 2,3,7,8-Tetrachlorodibenzo-p-dioxin. *Fundam. Appl. Toxicol.* **21**, 433–441 (1993).
159. Sampson, J. A. Ovarian Hematomas of Endometrial Type (Perforating Hemorrhagic Cysts of the Ovary) and Implantation Adenomas of Endometrial Type. *Bost. Med. Surg. J.* **186**, 445–456 (1922).
160. Sampson, J. A. Heterotopic or misplaced endometrial tissue. *Am. J. Obstet. Gynecol.* **10**, 649–664 (1925).
161. Halme, J., Hammond, M. G., Hulka, J. F., Raj, S. G. & Talbert, L. M. Retrograde menstruation in healthy women and in patients with endometriosis. *Obstet. Gynecol.* **64**, 151–154 (1984).
162. Sasson, I. E. & Taylor, H. S. Stem cells and the pathogenesis of endometriosis. in *Annals of the New York Academy of Sciences* vol. 1127 106–115 (Blackwell Publishing Inc., 2008).
163. Harirchian, P. *et al.* Lesion kinetics in a non-human primate model of endometriosis. *Hum. Reprod.* **27**, 2341–2351 (2012).
164. D'Hooghe, T. M. *et al.* Development of a model of retrograde menstruation in baboons (Papio anubis). *Fertil. Steril.* **62**, 635–638 (1994).
165. Burney, R. O. & Giudice, L. C. Pathogenesis and pathophysiology of endometriosis. *Fertility and Sterility* (2012) doi:10.1016/j.fertnstert.2012.06.029.
166. Sinaii, N., Cleary, S. D., Ballweg, M. L., Nieman, L. K. & Stratton, P. *High rates of autoimmune and endocrine disorders, fibromyalgia, chronic fatigue syndrome and atopic diseases among women with endometriosis: A survey analysis*. *Human Reproduction* vol. 17 https://academic.oup.com/humrep/article-abstract/17/10/2715/607769 (2002).
167. Kyama, C. M. *et al.* Role of cytokines in the endometrial-peritoneal cross-talk and development of endometriosis. *Frontiers in Bioscience - Elite* vol. 1 E 444–454 (2009).
168. Christodoulakos, G., Augoulea, A., Lambrinoudaki, I., Sioulas, V. & Creatsas, G. Pathogenesis of endometriosis: The role of defective 'immunosurveillance'. *Eur. J. Contracept. Reprod. Heal. Care* **12**, 194–202 (2007).
169. Ulukus, M. & Arici, A. Immunology of endometriosis. *Minerva Ginecologica* vol. 57 237–248 (2005).
170. Oosterlynck, D. J., Cornillie, F. J., Waer, M., Vandeputte, M. & Koninckx, P. R. Women with endometriosis show a defect in natural killer activity resulting in a decreased cytotoxicity to autologous endometrium. *Fertil. Steril.* **56**, 45–51 (1991).
171. Sikora, J., Mielczarek-Palacz, A. & Kondera-Anasz, Z. Role of Natural Killer Cell Activity in the Pathogenesis of Endometriosis. *Curr. Med. Chem.* **18**, 200–208 (2011).
172. Gruenwald, P. Origin of endometriosis from the mesenchyme of the celomic walls. *Am. J. Obstet. Gynecol.* **44**, 470–474 (1942).
173. Simsek, G. *et al.* An unusual cause of inguinal hernia in a male patient: Endometriosis. *Gut Liver* **6**, 284–285 (2012).
174. Figueira, P. G. M., Abrão, M. S., Krikun, G. & Taylor, H. Stem cells in endometrium and their role in the pathogenesis of endometriosis. *Ann. N. Y. Acad. Sci.* **1221**, 10–17 (2011).
175. Bontis, J. N. & Vavilis, D. T. Etiopathology of endometriosis. *Ann. N. Y. Acad. Sci.* **816**, 305–309 (1997).
176. Longo, L. D. Classic pages in obstetrics and gynecology. Aberrant portions of the müllerian duct found in an ovary: William Wood Russell Johns Hopkins Hospital Bulletin, vol. 10, pp. 8--10, 1899. *Am. J. Obstet. Gynecol.* **134**, 225–226 (1979).

177. Von Recklinghausen, F. Adenomyomas and cystadenomas of the wall of the uterus and tube: their origin as remnants of the wolffian body. *Wien Klin Wochenschr* **8**, 530 (1896).
178. Oliker, A. J. & Harris, A. E. Endometriosis of the bladder in a male patient. *J. Urol.* **106**, 858–859 (1971).
179. Schrodt, G. R., Alcorn, M. O. & Ibanez, J. Endometriosis of the male urinary system: A case report. *J. Urol.* **124**, 722–723 (1980).
180. Pinkert, T. C., Catlow, C. E. & Straus, R. Endometriosis of the urinary bladder in a man with prostatic carcinoma. *Cancer* **43**, 1562–1567 (1979).
181. Fukunaga, M. Paratesticular endometriosis in a man with a prolonged hormonal therapy for prostatic carcinoma. *Pathol. Res. Pract.* **208**, 59–61 (2012).
182. Young, R. H. & Scully, R. E. Testicular and paratesticular tumors and tumor-like lesions of ovarian common epithelial and Mullerian types. A report of four cases and review of the literature. *Am. J. Clin. Pathol.* **86**, 146–152 (1986).
183. Beckman, E. N., Pintado, S. O., Leonard, G. L. & Sternberg, W. H. Endometriosis of the prostate. *Am. J. Surg. Pathol.* **9**, 374–379 (1985).
184. Gargett, C. E. & Masuda, H. Adult stem cells in the endometrium. *Mol. Hum. Reprod.* **16**, 818–834 (2010).
185. Maruyama, T., Masuda, H., Ono, M., Kajitani, T. & Yoshimura, Y. Human uterine stem/progenitor cells: Their possible role in uterine physiology and pathology. *Reproduction* vol. 140 11–22 (2010).
186. Gargett, C. E. Uterine stem cells: What is the evidence? *Hum. Reprod. Update* **13**, 87–101 (2007).
187. Masuda, H. *et al.* Stem cell-like properties of the endometrial side population: Implication in endometrial regeneration. *PLoS One* **5**, e10387 (2010).
188. Leyendecker, G. *et al.* Uterine peristaltic activity and the development of endometriosis. in *Annals of the New York Academy of Sciences* vol. 1034 338–355 (New York Academy of Sciences, 2004).
189. Brosens, I., Gordts, S. & Benagiano, G. Endometriosis in adolescents is a hidden, progressive and severe disease that deserves attention, not just compassion. *Hum. Reprod.* **28**, 2026–2031 (2013).
190. Deane, J. A., Gualano, R. C. & Gargett, C. E. Regenerating endometrium from stem/progenitor cells: Is it abnormal in endometriosis, Asherman's syndrome and infertility? *Curr. Opin. Obstet. Gynecol.* **25**, 193–200 (2013).
191. Maruyama, T. & Yoshimura, Y. *Stem cell theory for the pathogenesis of endometriosis*. *Frontiers in Bioscience - Elite* vol. 4 E (2012).
192. Hoeger, K. M. & Guzick, D. S. Classification of endometriosis. *Obstet. Gynecol. Clin. North Am.* **24**, 347–359 (1997).
193. SAMPSON, J. A. Perforating Hemorrhagic (Chocolate) Cysts of the Ovary. *Arch. Surg.* **3**, 245 (1921).
194. Acosta, A. A. *et al.* A proposed classification of pelvic endometriosis. *Obstet. Gynecol.* **42**, 19–25 (1973).
195. Ammon, R. Biology and pathology of women: a manual of gynecology and obstetrics. Vol. 1. General part: 1. (1953).
196. Classification of endometriosis. *Fertil. Steril.* **32**, 633–634 (1979).
197. Andrews, W. C., Buttram, V. C. & Behrman, S. J. Revised American fertility society classification of endometriosis: 1985. *Fertil. Steril.* **44**, 7–8 (1985).
198. Johnson, N. P. *et al.* World endometriosis society consensus on the classification of endometriosis. *Hum. Reprod.* **32**, 315–324 (2017).
199. Adamson, G. D. Endometriosis classification: An update. *Curr. Opin. Obstet. Gynecol.* **23**, 213–220 (2011).
200. Tuttlies, F. *et al.* ENZIAN-Score, eine klassifikation der tief infiltrierenden endometriose. *Zentralblatt fur Gynakologie* vol. 127 275–281 (2005).
201. Haas, D. *et al.* Enzian classification: Does it correlate with clinical symptoms and the rASRM score? *Acta Obstet. Gynecol. Scand.* **92**, 562–566 (2013).

202. Adamson, G. D. & Pasta, D. J. Endometriosis fertility index: The new, validated endometriosis staging system. *Fertil. Steril.* **94**, 1609–1615 (2010).
203. Jörg Keckstein. Enzian scoring system for women with deep endometriosis. https://endometriose-sef.de/dateien/ENZIAN_2013_web.pdf (2013).
204. Vercellini, P. *et al.* Reproductive performance, pain recurrence and disease relapse after conservative surgical treatment for endometriosis: The predictive value of the current classification system. *Hum. Reprod.* **21**, 2679–2685 (2006).
205. Vercellini, P. *et al.* Association between endometriosis stage, lesion type, patient characteristics and severity of pelvic pain symptoms: A multivariate analysis of over 1000 patients. *Hum. Reprod.* **22**, 266–271 (2007).
206. Zondervan, K. T. *et al.* Endometriosis. *Nature Reviews Disease Primers* vol. 4 1–25 (2018).
207. Haas, D., Shebl, O., Shamiyeh, A. & Oppelt, P. The rASRM score and the Enzian classification for endometriosis: Their strengths and weaknesses. *Acta Obstet. Gynecol. Scand.* **92**, 3–7 (2013).
208. Haas, D. *et al.* Preoperative planning of surgery for deeply infiltrating endometriosis using the ENZIAN classification. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **166**, 99–103 (2013).
209. Adamson, G. D. & Pasta, D. J. Endometriosis fertility index: The new, validated endometriosis staging system. *Fertil. Steril.* **94**, 1609–1615 (2010).
210. Eskenazi, B. *et al.* Validation study of nonsurgical diagnosis of endometriosis. in *Fertility and Sterility* vol. 76 929–935 (2001).
211. Hudelist, G. *et al.* Transvaginal sonography vs. clinical examination in the preoperative diagnosis of deep infiltrating endometriosis. *Ultrasound Obstet. Gynecol.* **37**, 480–487 (2011).
212. Hudelist, G. *et al.* Combination of transvaginal sonography and clinical examination for preoperative diagnosis of pelvic endometriosis. *Hum. Reprod.* **24**, 1018–1024 (2009).
213. Bazot, M. *et al.* Diagnostic accuracy of physical examination, transvaginal sonography, rectal endoscopic sonography, and magnetic resonance imaging to diagnose deep infiltrating endometriosis. *Fertil. Steril.* **92**, 1825–1833 (2009).
214. Abrao, M. S. *et al.* Comparison between clinical examination, transvaginal sonography and magnetic resonance imaging for the diagnosis of deep endometriosis. *Hum. Reprod.* **22**, 3092–3097 (2007).
215. Cheewadhanaraks, S., Peeyananjarassri, K., Dhanaworavibul, K. & Liabsuetrakul, T. Positive predictive value of clinical diagnosis of endometriosis. *J. Med. Assoc. Thail.* **87**, 740–744 (2004).
216. Becker, C. M. *et al.* World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonisation Project: I. Surgical phenotype data collection in endometriosis research. *Fertility and Sterility* vol. 102 1213–1222 (2014).
217. Rahmioglu, N. *et al.* World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonization Project: III. Fluid biospecimen collection, processing, and storage in endometriosis research. *Fertility and Sterility* vol. 102 1233–1243 (2014).
218. Rogers, P. A. W. *et al.* Research Priorities for Endometriosis: Recommendations from a Global Consortium of Investigators in Endometriosis. in *Reproductive Sciences* vol. 24 202–226 (SAGE Publications Inc., 2017).
219. Guerriero, S. *et al.* Transvaginal ultrasonography combined with CA-125 plasma levels in the diagnosis of endometrioma. *Fertil. Steril.* **65**, 293–298 (1996).
220. Moore, J. *et al.* A systematic review of the accuracy of ultrasound in the diagnosis of endometriosis. *Ultrasound Obstet. Gynecol.* **20**, 630–634 (2002).
221. Dunselman, G. A. J. J. *et al.* ESHRE guideline: Management of women with endometriosis. *Hum. Reprod.* **29**, 400–412 (2014).
222. Van Holsbeke, C. *et al.* Endometriomas: Their ultrasound characteristics. *Ultrasound Obstet. Gynecol.* **35**, 730–740 (2010).
223. Bazot, M. *et al.* Accuracy of transvaginal sonography and rectal endoscopic sonography in the diagnosis of deep infiltrating endometriosis. *Ultrasound Obstet. Gynecol.* **30**, 994–1001 (2007).

224. Hudelist, G., Tuttlies, F., Rauter, G., Pucher, S. & Keckstein, J. Can transvaginal sonography predict infiltration depth in patients with deep infiltrating endometriosis of the rectum? *Hum. Reprod.* **24**, 1012–1017 (2009).
225. Piketty, M. *et al.* Preoperative work-up for patients with deeply infiltrating endometriosis: Transvaginal ultrasonography must definitely be the first-line imaging examination. *Hum. Reprod.* **24**, 602–607 (2009).
226. Stratton, P. *et al.* Diagnostic accuracy of laparoscopy, magnetic resonance imaging, and histopathologic examination for the detection of endometriosis. *Fertil. Steril.* **79**, 1078–1085 (2003).
227. Ascher, S. M. *et al.* Endometriosis: Appearance and detection with conventional and contrast-enhanced fat-suppressed spin-echo techniques. *J. Magn. Reson. Imaging* **5**, 251–257 (1995).
228. Outwater, E., Schiebler, M. L., Owen, R. S. & Schnall, M. D. Characterization of hemorrhagic adnexal lesions with MR imaging: Blinded reader study. *Radiology* **186**, 489–494 (1993).
229. Audebert, A. *et al.* Adolescent endometriosis: Report of a series of 55 cases with a focus on clinical presentation and long-term issues. *J. Minim. Invasive Gynecol.* **22**, 834–840 (2015).
230. Doltra, A. *et al.* Magnetic Resonance Imaging of Cardiovascular Fibrosis and Inflammation: From Clinical Practice to Animal Studies and Back Cardiovascular MRI View project Magnetic Resonance Imaging of Cardiovascular Fibrosis and Inflammation: From Clinical Practice to Ani. *Biomed Res. Int.* **676489**, 1–2 (2013).
231. Bourgioti, C. *et al.* MR imaging of endometriosis: Spectrum of disease. *Diagn. Interv. Imaging* **98**, 751–767 (2017).
232. Bazot, M., Gasner, A., Ballester, M. & Daraï, E. Value of thin-section oblique axial T2-weighted magnetic resonance images to assess uterosacral ligament endometriosis. *Hum. Reprod.* **26**, 346–353 (2011).
233. Chassang, M. *et al.* Utility of vaginal and rectal contrast medium in MRI for the detection of deep pelvic endometriosis. *Eur. Radiol.* **20**, 1003–1010 (2010).
234. Guerriero, S. *et al.* Transvaginal ultrasound vs magnetic resonance imaging for diagnosing deep infiltrating endometriosis: systematic review and meta-analysis. *Ultrasound Obstet. Gynecol.* **51**, 586–595 (2018).
235. Monte, G. Lo, Wenger, J. M., Petignat, P. & Marci, R. Role of imaging in endometriosis. *Cleve. Clin. J. Med.* **81**, 361–366 (2014).
236. Fasciani, A. *et al.* Endometriosis Index: A software-derived score to predict the presence and severity of the disease. *J. Endometr.* **2**, 79–86 (2010).
237. Yeung, P. *et al.* Development of a symptom-based, Screening tool for early-stage endometriosis in patients with chronic pelvic pain. *J. Endometr. Pelvic Pain Disord.* **6**, 174–189 (2014).
238. Forman, R. G. G., Robinson, J. N. N., Mehta, Z. & Barlow, D. H. H. Patient history as a simple predictor of pelvic pathology in subfertile women. *Hum. Reprod.* **8**, 53–55 (1993).
239. Calhaz-Jorge, C., Mol, B. W., Nunes, J. & Costa, A. P. Clinical predictive factors for endometriosis in a Portuguese infertile population. *Hum. Reprod.* **19**, 2126–2131 (2004).
240. Hackethal, A. *et al.* A structured questionnaire improves preoperative assessment of endometriosis patients: a retrospective analysis and prospective trial. *Arch. Gynecol. Obstet.* **284**, 1179–1188 (2011).
241. Ballard, K., Lane, H., Hudelist, G., Banerjee, S. & Wright, J. Can specific pain symptoms help in the diagnosis of endometriosis? A cohort study of women with chronic pelvic pain. *Fertil. Steril.* **94**, 20–27 (2010).
242. Nnoaham, K. E., Hummelshoj, L., Kennedy, S. H., Jenkinson, C. & Zondervan, K. T. Developing symptom-based predictive models of endometriosis as a clinical screening tool: Results from a multicenter study. *Fertil. Steril.* **98**, 692-701.e5 (2012).
243. *Endometriosis Screening & Education Kit*. (2012).
244. Saha, R., Marions, L. & Tornvall, P. Validity of self-reported endometriosis and endometriosis-related questions in a Swedish female twin cohort. *Fertil. Steril.* **107**, 174-178.e2 (2017).

245. Ashrafi, M., Jahanian Sadatmahalleh, S., Akhoond, M. R. & Talebi, M. Evaluation of risk factors associated with endometriosis in infertile women. *Int. J. Fertil. Steril.* **10**, 11–21 (2016).
246. Apostolopoulos, N. V., Alexandraki, K. I., Gorry, A. & Coker, A. Association between chronic pelvic pain symptoms and the presence of endometriosis. *Arch. Gynecol. Obstet.* **293**, 439–445 (2016).
247. Heitmann, R. J., Langan, K. L., Huang, R. R., Chow, G. E. & Burney, R. O. Premenstrual spotting of ≥2 days is strongly associated with histologically confirmed endometriosis in women with infertility. *Am. J. Obstet. Gynecol.* **211**, 358.e1-358.e6 (2014).
248. Paulson, J. D. & Paulson, J. N. Anterior vaginal wall tenderness (AVWT) as a physical symptom in chronic pelvic pain. *J. Soc. Laparoendosc. Surg.* **15**, 6–9 (2011).
249. Droz, J. & Howard, F. M. Use of the Short-Form McGill Pain Questionnaire as a Diagnostic Tool in Women with Chronic Pelvic Pain. *J. Minim. Invasive Gynecol.* **18**, 211–217 (2011).
250. Paulson, J. D. Correlation of anterior vaginal wall pain with endometriosis in infertile patients. *J. Reprod. Med. Obstet. Gynecol.* **54**, 145–150 (2009).
251. Flores, I. *et al.* Self-reported prevalence of endometriosis and its symptoms among Puerto Rican women. *Int. J. Gynecol. Obstet.* **100**, 257–261 (2008).
252. Griffiths, A. N., Koutsouridou, R. N. & Penketh, R. J. Predicting the presence of rectovaginal endometriosis from the clinical history: A retrospective observational study. *J. Obstet. Gynaecol. (Lahore).* **27**, 493–495 (2007).
253. Perelló, M. *et al.* Markers of deep infiltrating endometriosis in patients with ovarian endometrioma: a predictive model. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **209**, 55–60 (2017).
254. Lafay Pillet, M. C. *et al.* A clinical score can predict associated deep infiltrating endometriosis before surgery for an endometrioma. *Hum. Reprod.* **29**, 1666–1676 (2014).
255. Barcellos, M. B., Lasmar, B. & Lasmar, R. Agreement between the preoperative findings and the operative diagnosis in patients with deep endometriosis. *Arch. Gynecol. Obstet.* **293**, 845–850 (2016).
256. Lasmar, R. B., Lasmar, B. P. & Pillar, C. Diagram to map the locations of endometriosis. *Int. J. Gynecol. Obstet.* **118**, 42–46 (2012).
257. Fernando, S. *et al.* Reliability of visual diagnosis of endometriosis. *J. Minim. Invasive Gynecol.* **20**, 783–789 (2013).
258. Stegmann, B. J. *et al.* Using location, color, size, and depth to characterize and identify endometriosis lesions in a cohort of 133 women. *Fertil. Steril.* **89**, 1632–1636 (2008).
259. Kazanegra, R., Zaritsky, E., Lathi, R. B., Clopton, P. & Nezhat, C. Diagnosis of Stage I Endometriosis: Comparing Visual Inspection to Histologic Biopsy Specimen. *J. Minim. Invasive Gynecol.* **15**, 176–180 (2008).
260. Wykes, C. B., Clark, T. J. & Khan, K. S. Accuracy of laparoscopy in the diagnosis of endometriosis: A systematic quantitative review. *BJOG An Int. J. Obstet. Gynaecol.* **111**, 1204–1212 (2004).
261. Coutinho, L. M., Ferreira, M. C., Rocha, A. L. L., Carneiro, M. M. & Reis, F. M. *New biomarkers in endometriosis*. *Advances in Clinical Chemistry* vol. 89 (Academic Press Inc., 2019).
262. Bedaiwy, M. A. & Falcone, T. Laboratory testing for endometriosis. *Clinica Chimica Acta* vol. 340 41–56 (2004).
263. Brosens, I., Puttemans, P., Campo, R., Gordts, S. & Brosens, J. Non-invasive methods of diagnosis of endometriosis. *Curr. Opin. Obstet. Gynecol.* **15**, 519–522 (2003).
264. Othman, E. E.-D. R. D. R. *et al.* Serum cytokines as biomarkers for nonsurgical prediction of endometriosis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **137**, 240–246 (2008).
265. Yang, W. C. V. *et al.* Serum and endometrial markers. *Best Pract. Res. Clin. Obstet. Gynaecol.* **18**, 305–318 (2004).
266. Fassbender, A., Burney, R. O., O, D. F., D'Hooghe, T. & Giudice, L. Update on Biomarkers for the Detection of Endometriosis. *Biomed Res. Int.* **2015**, (2015).

267. D'Hooghe, T. M. *et al.* Why we need a noninvasive diagnostic test for minimal to mild endometriosis with a high sensitivity. *Gynecol. Obstet. Invest.* **62**, 136–138 (2006).
268. May, K. E. *et al.* Peripheral biomarkers of endometriosis: A systematic review. *Hum. Reprod. Update* **16**, 651–674 (2010).
269. Nisenblat, V. *et al. Blood biomarkers for the non-invasive diagnosis of endometriosis*. *Cochrane Database of Systematic Reviews* vol. 2016 CD012179 (John Wiley and Sons Ltd, 2016).
270. Gupta, D. *et al.* Endometrial biomarkers for the non-invasive diagnosis of endometriosis. *Cochrane Database of Systematic Reviews* vol. 2016 (2016).
271. Gao, Y. *et al.* Seven Hormonal Biomarkers for Diagnosing Endometriosis: Meta-Analysis and Adjusted Indirect Comparison of Diagnostic Test Accuracy. *Journal of Minimally Invasive Gynecology* vol. 26 1026-1035.e4 (2019).
272. Yin, B. W. T., Dnistrian, A. & Lloyd, K. O. Ovarian cancer antigen CA125 is encoded by the MUC16 mucin gene. *Int. J. Cancer* **98**, 737–740 (2002).
273. Yin, B. W. T. & Lloyd, K. O. Molecular cloning of the CA125 ovarian cancer antigen: Identification as a new mucin, MUC16. *J. Biol. Chem.* **276**, 27371–27375 (2001).
274. Niloff, J. M. *et al.* Elevation of serum CA125 in carcinomas of the fallopian tube, endometrium, and endocervix. *Am. J. Obstet. Gynecol.* **148**, 1057–1058 (1984).
275. Kabawat, S. E. *et al.* Tissue distribution of a coelomic- epithelium-related antigen recognized by the monoclonal antibody OC125. *Int. J. Gynecol. Pathol.* **2**, 275–285 (1983).
276. Bast, J. *et al.* CA 125: The past and the future. *International Journal of Biological Markers* vol. 13 179–187 (1998).
277. Bast, R. C. *et al.* A Radioimmunoassay Using a Monoclonal Antibody to Monitor the Course of Epithelial Ovarian Cancer. *N. Engl. J. Med.* **309**, 883–887 (1983).
278. Scambia, G. *et al.* Measurement of a monoclonal-antibody-defined antigen (90K) in the sera of patients with ovarian cancer. *Anticancer Res.* **8**, 761–764 (1988).
279. Bast, R. C. *et al.* New tumor markers: CA125 and beyond. *Int. J. Gynecol. Cancer* **15**, 274–281 (2005).
280. Meden, H. & Fattani-Meiodi, A. CA 125 in benign gynecological conditions. *International Journal of Biological Markers* vol. 13 231–237 (1998).
281. Hirsch, M., Duffy, J. M. N., Davis, C. J., Nieves Plana, M. & Khan, K. S. Diagnostic accuracy of cancer antigen 125 for endometriosis: a systematic review and meta-analysis. *BJOG An Int. J. Obstet. Gynaecol.* **123**, 1761–1768 (2016).
282. Park, Y., Kim, Y., Lee, E. Y., Lee, J. H. & Kim, H. S. Reference ranges for HE4 and CA125 in a large Asian population by automated assays and diagnostic performances for ovarian cancer. *Int. J. Cancer* **130**, 1136–1144 (2012).
283. Barbieri, R. L. *et al.* Elevated serum concentrations of CA-125 in patients with advanced endometriosis. *Fertil. Steril.* **45**, 630–634 (1986).
284. Patton, P. E., Field, C. S., Harms, R. W. & Coulam, C. B. CA-125 levels in endometriosis. *Fertil. Steril.* **45**, 770–773 (1986).
285. Giudice, L. C., Jacobs, A., Pineda, J., Bell, C. E. & Lippmann, L. Serum levels of CA-125 in patients with endometriosis: A preliminary report. *Fertil. Steril.* **45**, 876–878 (1986).
286. Moretuzzo, R. W. *et al.* Serum and peritoneal lavage fluid CA-125 levels in endometriosis. *Fertil. Steril.* **50**, 430–433 (1988).
287. Fedele, L., Vercellini, P., Arcaini, L., Grazia da Dalt, M. & Candiani, G. B. CA 125 in serum, peritoneal fluid, active lesions, and endometrium of patients with endometriosis. *Am. J. Obstet. Gynecol.* **158**, 166–170 (1988).
288. Lanzone, A. *et al.* Serum CA-125 levels in the diagnosis and management of endometriosis. *J. Reprod. Med. Obstet. Gynecol.* **36**, 603–607 (1991).
289. Hornstein, M. D., Thomas, P. P., Gleason, R. E. & Barbieri, R. L. Menstrual cyclicity of CA-125 in patients with endometriosis. *Fertil. Steril.* **58**, 279–283 (1992).

290. O'Shaughnessy, A., Check, J. H., Nowroozi, K. & Lurie, D. CA 125 levels measured in different phases of the menstrual cycle in screening for endometriosis. *Obstet. Gynecol.* **81**, 99–103 (1993).
291. Chen, F. P., Soong, Y. K., Lee, N. & Lo, S. K. The use of serum CA-125 as a marker for endometriosis in patients with dysmenorrhea for monitoring therapy and for recurrence of endometriosis. *Acta Obstet. Gynecol. Scand.* **77**, 665 (1998).
292. Santulli, P. *et al.* Increased Serum Cancer Antigen-125 Is a Marker for Severity of Deep Endometriosis. *J. Minim. Invasive Gynecol.* **22**, 275–284 (2015).
293. Oliveira, M. A. P., Raymundo, T. S., Soares, L. C., Pereira, T. R. D. & Demôro, A. V. E. How to Use CA-125 more effectively in the diagnosis of deep endometriosis. *Biomed Res. Int.* **2017**, (2017).
294. Kafali, H., Artuc, H. & Demir, N. Use of CA125 fluctuation during the menstrual cycle as a tool in the clinical diagnosis of endometriosis; a preliminary report. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **116**, 85–88 (2004).
295. Somigliana, E. *et al.* Use of the concomitant serum dosage of CA 125, CA 19-9 and interleukin-6 to detect the presence of endometriosis. Results from a series of reproductive age women undergoing laparoscopic surgery for benign gynaecological conditions. *Hum. Reprod.* **19**, 1871–1876 (2004).
296. Ferrero, S. *et al.* Haptoglobin β chain isoforms in the plasma and peritoneal fluid of women with endometriosis. *Fertil. Steril.* **83**, 1536–1543 (2005).
297. Tortoriello, D. V., Sidis, Y., Holtzman, D. A., Holmes, W. E. & Schneyer, A. L. Human follistatin-related protein: A structural homologue of follistatin with nuclear localization. *Endocrinology* **142**, 3426–3434 (2001).
298. Ueno, N. *et al.* Isolation and partial characterization of follistatin: a single-chain Mr 35,000 monomeric protein that inhibits the release of follicle-stimulating hormone. *Proc. Natl. Acad. Sci. U. S. A.* **84**, 8282–8286 (1987).
299. Torres, P. B. *et al.* Deranged expression of follistatin and follistatin-like protein in women with ovarian endometriosis. *Fertil. Steril.* **88**, 200–205 (2007).
300. Florio, P. *et al.* High serum follistatin levels in women with ovarian endometriosis. *Hum. Reprod.* **24**, 2600–2606 (2009).
301. Reis, F. M. *et al.* Diagnostic value of serum activin A and follistatin levels in women with peritoneal, ovarian and deep infiltrating endometriosis. *Hum. Reprod.* **27**, 1445–1450 (2012).
302. Borrelli, G. M., Abrão, M. S. & Mechsner, S. Can chemokines be used as biomarkers for endometriosis? A systematic review. *Hum. Reprod.* **29**, 253–266 (2014).
303. Luisi, S., Pinzauti, S., Regini, C. & Petraglia, F. Serum markers for the noninvasive diagnosis of endometriosis. *Women's Heal.* **11**, 603–610 (2015).
304. Martínez, S. *et al.* Serum interleukin-6 levels are elevated in women with minimal-mild endometriosis. *Hum. Reprod.* **22**, 836–842 (2007).
305. Andreoli, C. G. *et al.* T helper (Th)1, Th2, and Th17 interleukin pathways in infertile patients with minimal/mild endometriosis. *Fertil. Steril.* **95**, 2477–2480 (2011).
306. Kilic, S. H. *et al.* Follicular fluid vascular endothelial growth factor and tumour necrosis factor α concentrations in patients with endometriosis undergoing ICSI. *Reprod. Biomed. Online* **15**, 316–320 (2007).
307. Bedaiwy, M. A. *et al. Prediction of endometriosis with serum and peritoneal fluid markers: A prospective controlled trial*. *Human Reproduction* vol. 17 (2002).
308. Richter, O. N., Dorn, C., Rösing, B., Flaskamp, C. & Ulrich, U. Tumor necrosis factor alpha secretion by peritoneal macrophages in patients with endometriosis. *Arch. Gynecol. Obstet.* **271**, 143–147 (2005).
309. Xavier, P. *et al.* Serum levels of VEGF and TNF-a and their association with C-reactive protein in patients with endometriosis. *Arch. Gynecol. Obstet.* **273**, 227–231 (2006).
310. Vodolazkaia, A. *et al.* A high sensitivity assay is more accurate than a classical assay for the measurement of plasma CRP levels in endometriosis. *Reprod. Biol. Endocrinol.* **9**, 113 (2011).

311. Lermann, J. *et al.* Evaluation of high-sensitivity C-reactive protein in comparison with C-reactive protein as biochemical serum markers in women with endometriosis. *Fertil. Steril.* **93**, 2125–2129 (2010).
312. Garcia-Velasco, J. A. *et al.* Is endometrial receptivity transcriptomics affected in women with endometriosis? A pilot study. *Reprod. Biomed. Online* **31**, 647–654 (2015).
313. Talbi, S. *et al.* Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. *Endocrinology* **147**, 1097–1121 (2006).
314. Sampson, J. A. Metastatic or Embolic Endometriosis, due to the Menstrual Dissemination of Endometrial Tissue into the Venous Circulation. *Am. J. Pathol.* **3**, 93-110.43 (1927).
315. Naqvi, H., Ilagan, Y., Krikun, G. & Taylor, H. S. Altered genome-wide methylation in endometriosis. *Reprod. Sci.* **21**, 1237–1243 (2014).
316. Burney, R. O. *et al.* MicroRNA expression profiling of eutopic secretory endometrium in women with versus without endometriosis. *Mol. Hum. Reprod.* **15**, 625–631 (2009).
317. Hasin, Y., Seldin, M. & Lusis, A. Multi-omics approaches to disease. *Genome Biology* vol. 18 1–15 (2017).
318. LaFramboise, T. Single nucleotide polymorphism arrays: a decade of biological, computational and technological advances. *Nucleic Acids Res.* **37**, 4181–4193 (2009).
319. Manolio, T. A. Genomewide association studies and assessment of the risk of disease. *N. Engl. J. Med.* **363**, 166–176 (2010).
320. Begum, F., Ghosh, D., Tseng, G. C. & Feingold, E. Comprehensive literature review and statistical considerations for GWAS meta-analysis. *Nucleic Acids Res.* **40**, 3777–3784 (2012).
321. Saare, M. *et al.* OMICs studies and endometriosis biomarker identification. in *Biomarkers for Endometriosis: State of the Art* 227–258 (Springer International Publishing, 2017). doi:10.1007/978-3-319-59856-7_12.
322. Vicente-Muñoz, S. *et al.* Nuclear magnetic resonance metabolomic profiling of urine provides a noninvasive alternative to the identification of biomarkers associated with endometriosis. *Fertil. Steril.* **104**, 1202–1209 (2015).
323. Ametzazurra, A. *et al.* Endometrial fluid is a specific and non-invasive biological sample for protein biomarker identification in endometriosis. *Hum. Reprod.* **24**, 954–965 (2009).
324. Hathout, Y. Approaches to the study of the cell secretome. *Expert Review of Proteomics* vol. 4 239–248 (2007).
325. Kiemer, L. & Cesareni, G. Comparative interactomics: comparing apples and pears? *Trends in Biotechnology* vol. 25 448–454 (2007).
326. Ozdemir, V. *et al.* Risk Assessment and Communication Tools for Genotype Associations with Multifactorial Phenotypes: The Concept of 'edge Effect' and Cultivating an Ethical Bridge between Omics Innovations and Society. *OMICS A Journal of Integrative Biology* vol. 13 43–61 (2009).
327. Guo, S. W. *et al.* Genomic alterations in the endometrium may be a proximate cause for endometriosis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **116**, 89–99 (2004).
328. Li, X. *et al.* Whole-exome sequencing of endometriosis identifies frequent alterations in genes involved in cell adhesion and chromatin-remodeling complexes. *Hum. Mol. Genet.* **23**, 6008–6021 (2014).
329. Sahar Houshdaran *et al.* Aberrant Endometrial DNA Methylome and Associated Gene Expression in Women with Endometriosis1. *Biol. Reprod.* **95**, (2016).
330. Saare, M. *et al.* The influence of menstrual cycle and endometriosis on endometrial methylome. *Clin. Epigenetics* **8**, 1–10 (2016).
331. Shi, X. Y., Gu, L., Chen, J., Guo, X. R. & Shi, Y. L. Downregulation of miR-183 inhibits apoptosis and enhances the invasive potential of endometrial stromal cells in endometriosis. *Int. J. Mol. Med.* **33**, 59–67 (2014).

332. Fassbender, A. *et al.* Combined mRNA microarray and proteomic analysis of eutopic endometrium of women with and without endometriosis. *Hum. Reprod.* **27**, 2020–2029 (2012).
333. Kao, L. C. *et al.* Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease. Based implantation failure and infertility. *Endocrinology* **144**, 2870–2881 (2003).
334. Aghajanova, L. & Giudice, L. C. Molecular evidence for differences in endometrium in severe versus mild endometriosis. *Reprod. Sci.* **18**, 229–251 (2011).
335. Zhou, M. *et al.* MiR-196a overexpression activates the MEK/ERK signal and represses the progesterone receptor and decidualization in eutopic endometrium from women with endometriosis. *Hum. Reprod.* **31**, 2598–2608 (2016).
336. Suryawanshi, S. *et al.* Complement pathway is frequently altered in endometriosis and endometriosis-associated ovarian cancer. *Clin. Cancer Res.* **20**, 6163–6174 (2014).
337. Ahn, S. H. *et al.* Immune-inflammation gene signatures in endometriosis patients. *Fertil. Steril.* **106**, 1420-1431.e7 (2016).
338. Matsuzaki, S. *et al.* DNA microarray analysis of gene expression in eutopic endometrium from patients with deep endometriosis using laser capture microdissection. *Fertil. Steril.* **84**, 1180–1190 (2005).
339. Sherwin, J. R. A. *et al.* Global gene analysis of late secretory phase, eutopic endometrium does not provide the basis for a minimally invasive test of endometriosis. *Hum. Reprod.* **23**, 1063–1068 (2008).
340. Burney, R. O. *et al.* Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. *Endocrinology* **148**, 3814–3826 (2007).
341. Absenger, Y. *et al.* Cyr61, a deregulated gene in endometriosis. *Mol. Hum. Reprod.* **10**, 399–407 (2004).
342. Tamaresis, J. S. *et al.* Molecular classification of endometriosis and disease stage using high-dimensional genomic data. *Endocrinology* **155**, 4986–4999 (2014).
343. Wang, Y., Li, Y., Yang, Z., Liu, K. & Wang, D. Genome-wide microarray analysis of long non-coding RNAs in Eutopic secretory endometrium with endometriosis. *Cell. Physiol. Biochem.* **37**, 2231–2245 (2015).
344. Laudanski, P. *et al.* MicroRNAs expression profiling of eutopic proliferative endometrium in women with ovarian endometriosis. *Reprod. Biol. Endocrinol.* **11**, (2013).
345. Laudanski, P. *et al.* Profiling of selected microRNAs in proliferative eutopic endometrium of women with ovarian endometriosis. *Biomed Res. Int.* **2015**, (2015).
346. Braza-Boïls, A. *et al.* MicroRNA expression profile in endometriosis: Its relation to angiogenesis and fibrinolytic factors. *Hum. Reprod.* **29**, 978–988 (2014).
347. Fassbender, A. *et al.* TRIzol treatment of secretory phase endometrium allows combined proteomic and mRNA microarray analysis of the same sample in women with and without endometriosis. *Reprod. Biol. Endocrinol.* **8**, (2010).
348. Rai, P., Kota, V., Deendayal, M. & Shivaji, S. Differential proteome profiling of eutopic endometrium from women with endometriosis to understand etiology of endometriosis. *J. Proteome Res.* **9**, 4407–4419 (2010).
349. Fowler, P. A. *et al.* An investigation of the effects of endometriosis on the proteome of human eutopic endometrium: A heterogeneous tissue with a complex disease. *Proteomics* **7**, 130–142 (2007).
350. Ding, X., Wang, L., Ren, Y. & Zheng, W. Detection of mitochondrial biomarkers in eutopic endometria of endometriosis using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry. *Fertil. Steril.* **94**, 2528–2530 (2010).
351. Kyama, C. M. *et al.* Evaluation of endometrial biomarkers for semi-invasive diagnosis of endometriosis. *Fertil. Steril.* **95**, (2011).

352. Wang, L. *et al.* Identification biomarkers of eutopic endometrium in endometriosis using artificial neural networks and protein fingerprinting. *Fertil. Steril.* **93**, 2460–2462 (2010).
353. Zhang, H. *et al.* Use of proteomic analysis of endometriosis to identify different protein expression in patients with endometriosis versus normal controls. *Fertil. Steril.* **86**, 274–282 (2006).
354. ten Have, S., Fraser, I., Markham, R., Lam, A. & Matsumoto, I. Proteomic analysis of protein expression in the eutopic endometrium of women with endometriosis. *Proteomics - Clin. Appl.* **1**, 1243–1251 (2007).
355. Stephens, A. N. *et al.* Post-translational modifications and protein-specific isoforms in endometriosis revealed by 2D DIGE. *J. Proteome Res.* **9**, 2438–2449 (2010).
356. Gogusev, J. *et al.* Detection of DNA copy number changes in human endometriosis by comparative genomic hybridization. *Hum. Genet.* **105**, 444–451 (1999).
357. Wu, Y. *et al.* Genomic alterations in ectopic and eutopic endometria of women with endometriosis. *Gynecol. Obstet. Invest.* **62**, 148–159 (2006).
358. Veiga-Castelli, L. C. *et al.* Genomic alterations detected by comparative genomic hybridization in ovarian endometriomas. *Brazilian J. Med. Biol. Res.* **43**, 799–805 (2010).
359. Saare, M. *et al.* No evidence of somatic DNA copy number alterations in eutopic and ectopic endometrial tissue in endometriosis. *Hum. Reprod.* **27**, 1857–1864 (2012).
360. Yang, W., Zhang, Y., Fu, F. & Li, R. High-resolution array-comparative genomic hybridization profiling reveals 20q13.33 alterations associated with ovarian endometriosis. *Gynecol. Endocrinol.* **29**, 603–607 (2013).
361. Silveira, C. G. T. *et al.* Common chromosomal imbalances and stemness-related protein expression markers in endometriotic lesions from different anatomical sites: The potential role of stem cells. *Hum. Reprod.* **27**, 3187–3197 (2012).
362. Zafrakas, M. *et al.* Genome-wide microarray gene expression, array-CGH analysis, and telomerase activity in advanced ovarian endometriosis: A high degree of differentiation rather than malignant potential. *Int. J. Mol. Med.* **21**, 335–344 (2008).
363. Borghese, B. *et al.* Research resource: Genome-wide profiling of methylated promoters in endometriosis reveals a subtelomeric location of hypermethylation. *Mol. Endocrinol.* **24**, 1872–1885 (2010).
364. Yamagata, Y. *et al.* Genome-wide DNA methylation profiling in cultured eutopic and ectopic endometrial stromal cells. *PLoS One* **9**, (2014).
365. Dyson, M. T. *et al.* Genome-Wide DNA Methylation Analysis Predicts an Epigenetic Switch for GATA Factor Expression in Endometriosis. *PLoS Genet.* **10**, e1004158 (2014).
366. Hawkins, S. M. *et al.* Functional microRNA involved in endometriosis. *Mol. Endocrinol.* **25**, 821–832 (2011).
367. Hever, A. *et al.* Human endometriosis is associated with plasma cells and overexpression of B lymphocyte stimulator. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 12451–12456 (2007).
368. Monsivais, D. *et al.* Estrogen receptor β regulates endometriotic cell survival through serum and glucocorticoid-regulated kinase activation. *Fertil. Steril.* **105**, 1266–1273 (2016).
369. Crispi, S. *et al.* Transcriptional profiling of endometriosis tissues identifies genes related to organogenesis defects. *J. Cell. Physiol.* **228**, 1927–1934 (2013).
370. Sun, P. R., Jia, S. Z., Lin, H., Leng, J. H. & Lang, J. H. Genome-wide profiling of long noncoding ribonucleic acid expression patterns in ovarian endometriosis by microarray. *Fertil. Steril.* **101**, (2014).
371. Eyster, K. M., Boles, A. L., Brannian, J. D. & Hansen, K. A. DNA microarray analysis of gene expression markers of endometriosis. *Fertil. Steril.* **77**, 38–42 (2002).
372. Mettler, L. *et al.* Comparison of c-DNA microarray analysis of gene expression between eutopic endometrium and ectopic endometrium (endometriosis). *J. Assist. Reprod. Genet.* **24**, 249–258 (2007).

373. Lebovic, D. I., Baldocchi, R. A., Mueller, M. D. & Taylor, R. N. Altered expression of a cell-cycle suppressor gene, Tob-1, in endometriotic cells by cDNA array analyses. in *Fertility and Sterility* vol. 78 849–854 (Elsevier Inc., 2002).
374. Konno, R. *et al.* Role of immunoreactions and mast cells in pathogenesis of human endometriosis--morphologic study and gene expression analysis. *Hum. cell Off. J. Hum. Cell Res. Soc.* **16**, 141–149 (2003).
375. Matsuzaki, S. *et al.* DNA microarray analysis of gene expression profiles in deep endometriosis using laser capture microdissection. *Mol. Hum. Reprod.* **10**, 719–728 (2004).
376. Hu, W. P., Sun, K. T. & Zháo, Y. Endometriosis-specific genes identified by real-time reverse transcription-polymerase chain reaction expression profiling of endometriosis Versus autologous uterine endometrium. *J. Clin. Endocrinol. Metab.* **91**, 228–238 (2006).
377. Arimoto, T. *et al.* Genome-wide cDNA microarray analysis of gene-expression profiles involved in ovarian endometriosis. *Int. J. Oncol.* **22**, 551–560 (2003).
378. Eyster, K. M., Klinkova, O., Kennedy, V. & Hansen, K. A. Whole genome deoxyribonucleic acid microarray analysis of gene expression in ectopic versus eutopic endometrium. *Fertil. Steril.* **88**, 1505–1533 (2007).
379. Wu, Y. *et al.* Transcriptional characterizations of differences between eutopic and ectopic endometrium. *Endocrinology* **147**, 232–246 (2006).
380. Borghese, B. *et al.* Gene expression profile for ectopic versus eutopic endometrium provides new insights into endometriosis oncogenic potential. *Mol. Endocrinol.* **22**, 2557–2562 (2008).
381. Khan, M. A., Sengupta, J., Mittal, S. & Ghosh, D. Genome-wide expressions in autologous eutopic and ectopic endometrium of fertile women with endometriosis. *Reprod. Biol. Endocrinol.* **10**, 84 (2012).
382. Abe, W. *et al.* MiR-196b targets c-myc and Bcl-2 expression, inhibits proliferation and induces apoptosis in endometriotic stromal cells. *Hum. Reprod.* **28**, 750–761 (2013).
383. Yang, R. Q. *et al.* Microarray analysis of microRNA deregulation and angiogenesis-related proteins in endometriosis. *Genet. Mol. Res.* **15**, (2016).
384. Saare, M. *et al.* High-throughput sequencing approach uncovers the miRNome of peritoneal endometriotic lesions and adjacent healthy tissues. *PLoS One* **9**, (2014).
385. Teague, E. M. C. O. *et al.* MicroRNA-regulated pathways associated with endometriosis. *Mol. Endocrinol.* **23**, 265–275 (2009).
386. Filigheddu, N. *et al.* Differential expression of micrornas between eutopic and ectopic endometrium in ovarian endometriosis. *J. Biomed. Biotechnol.* **2010**, (2010).
387. Vehmas, A. P. *et al.* Ovarian endometriosis signatures established through discovery and directed mass spectrometry analysis. *J. Proteome Res.* **13**, 4983–4994 (2014).
388. Chehna-Patel, N., Sachdeva, G., Gajbhiye, R., Warty, N. & Khole, V. 'Spot'-ting differences between the ectopic and eutopic endometrium of endometriosis patients. *Fertil. Steril.* **94**, (2010).
389. Kasvandik, S. *et al.* Deep Quantitative Proteomics Reveals Extensive Metabolic Reprogramming and Cancer-Like Changes of Ectopic Endometriotic Stromal Cells. *J. Proteome Res.* **15**, 572–584 (2016).
390. Marianowski, P., Szymusik, I., Malejczyk, J., Hibner, M. & Wielgos, M. Proteomic analysis of eutopic and ectopic endometriotic tissues based on isobaric peptide tags for relative and absolute quantification (iTRAQ) method. *Neuro Endocrinol. Lett.* **34**, 717–721 (2013).
391. Kyama, C. M. *et al.* ProteinChip* * ProteinChip (Ciphergen Biosystems, Inc., Fremont, CA). technology is a useful method in the pathogenesis and diagnosis of endometriosis: a preliminary study. *Fertil. Steril.* **86**, 203–209 (2006).
392. Mafra, F. *et al.* Copy number variation analysis reveals additional variants contributing to endometriosis development. *J. Assist. Reprod. Genet.* **34**, 117–124 (2017).
393. Chettier, R., Ward, K. & Albertsen, H. M. Endometriosis is associated with rare copy number variants. *PLoS One* **9**, (2014).

394. Steinthorsdottir, V. *et al.* Common variants upstream of KDR encoding VEGFR2 and in TTC39B associate with endometriosis. *Nat. Commun.* **7**, (2016).
395. Albertsen, H. M., Chettier, R., Farrington, P. & Ward, K. Genome-Wide Association Study Link Novel Loci to Endometriosis. *PLoS One* **8**, (2013).
396. Painter, J. N. *et al.* Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis. *Nat. Genet.* **43**, 51–54 (2011).
397. Adachi, S. *et al.* Meta-analysis of genome-wide association scans for genetic susceptibility to endometriosis in Japanese population. *J. Hum. Genet.* **55**, 816–821 (2010).
398. Uno, S. *et al.* A genome-wide association study identifies genetic variants in the CDKN2BAS locus associated with endometriosis in Japanese. *Nat. Genet.* **42**, 707–710 (2010).
399. Cosar, E. *et al.* Serum microRNAs as diagnostic markers of endometriosis: a comprehensive array-based analysis. *Fertil. Steril.* **106**, 402–409 (2016).
400. Wang, L. *et al.* Analysis of Serum microRNA Profile by Solexa Sequencing in Women with Endometriosis. *Reprod. Sci.* **23**, 1359–1370 (2016).
401. Wang, W. T., Zhao, Y. N., Han, B. W., Hong, S. J. & Chen, Y. Q. Circulating microRNAs identified in a genome-wide serum microRNA expression analysis as noninvasive biomarkers for endometriosis. *J. Clin. Endocrinol. Metab.* **98**, 281–289 (2013).
402. Jia, S. Z., Yang, Y., Lang, J., Sun, P. & Leng, J. Plasma miR-17-5p, miR-20a and miR-22 are down-regulated in women with endometriosis. *Hum. Reprod.* **28**, 322–330 (2013).
403. Hsu, C. Y. *et al.* MiRNA-199a-5p regulates VEGFA in endometrial mesenchymal stem cells and contributes to the pathogenesis of endometriosis. *J. Pathol.* **232**, 330–343 (2014).
404. Suryawanshi, S. *et al.* Plasma MicroRNAs as novel biomarkers for endometriosis and endometriosis-associated ovarian cancer. *Clin. Cancer Res.* **19**, 1213–1224 (2013).
405. Gajbhiye, R. *et al.* Identification and validation of novel serum markers for early diagnosis of endometriosis. *Hum. Reprod.* **27**, 408–417 (2012).
406. Faserl, K. *et al.* Polymorphism in vitamin D-binding protein as a genetic risk factor in the pathogenesis of endometriosis. *J. Clin. Endocrinol. Metab.* **96**, (2011).
407. Seeber, B. *et al.* Proteomic analysis of serum yields six candidate proteins that are differentially regulated in a subset of women with endometriosis. *Fertil. Steril.* **93**, 2137–2144 (2010).
408. Wang, L. *et al.* Artificial neural networks combined with surface-enhanced laser desorption/ionization mass spectra distinguish endometriosis from healthy population. *Fertil. Steril.* **88**, 1700–1702 (2007).
409. Dutta, M. *et al.* Investigation of serum proteome alterations in human endometriosis. *J. Proteomics* **114**, 182–196 (2015).
410. Tokushige, N. *et al.* Discovery of a novel biomarker in the urine in women with endometriosis. *Fertil. Steril.* **95**, 46–49 (2011).
411. Hwang, J. H. *et al.* Identification of biomarkers for endometriosis in eutopic endometrial cells from patients with endometriosis using a proteomics approach. *Mol. Med. Rep.* **8**, 183–188 (2013).
412. Siciliano, R. A. *et al.* Rapid peptidomic profiling of peritoneal fluid by MALDI-TOF mass spectrometry for the identification of biomarkers of endometriosis. *Gynecol. Endocrinol.* **30**, 872–876 (2014).
413. Ferrero, S. *et al.* Proteomic analysis of peritoneal fluid in women with endometriosis. *J. Proteome Res.* **6**, 3402–3411 (2007).
414. Ferrero, S. *et al.* Peritoneal fluid proteome in women with different ASRM stages of endometriosis. *Gynecol. Endocrinol.* **24**, 433–441 (2008).
415. Ferrero, S. *et al.* Proteomic analysis of peritoneal fluid in fertile and infertile women with endometriosis. *J. Reprod. Med. Obstet. Gynecol.* **54**, 32–40 (2009).
416. Wölfler, M. M. *et al.* Two-dimensional gel electrophoresis in peritoneal fluid samples identifies differential protein regulation in patients suffering from peritoneal or ovarian endometriosis. *Fertil. Steril.* **95**, 2764–2768 (2011).

417. Cho, S. H. *et al.* Urinary vitamin D-binding protein is elevated in patients with endometriosis. *Hum. Reprod.* **27**, 515–522 (2012).
418. Zheng, N., Pan, C. & Liu, W. New serum biomarkers for detection of endometriosis using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J. Int. Med. Res.* **39**, 1184–1192 (2011).
419. Williams, K. E. *et al.* Urine, peritoneal fluid and omental fat proteomes of reproductive age women: Endometriosis-related changes and associations with endocrine disrupting chemicals. *J. Proteomics* **113**, 194–205 (2015).
420. Fassbender, A. *et al.* Proteomics analysis of plasma for early diagnosis of endometriosis. *Obstet. Gynecol.* **119**, 276–285 (2012).
421. El-Kasti, M. M. *et al.* Urinary peptide profiling identifies a panel of putative biomarkers for diagnosing and staging endometriosis. *Fertil. Steril.* **95**, 1261-1266.e6 (2011).
422. Wang, L., Ding, X. Y., Yu, J. K., Zhang, S. Z. & Zheng, W. Biomarkers of peritoneal fluid in endometriosis identified by surface-enhanced laser desorption/ionization time-of-flight. *Clin. Exp. Obstet. Gynecol.* **41**, 72–74 (2014).
423. Liu, H., Lang, J., Zhou, Q., Shan, D. & Li, Q. Detection of endometriosis with the use of plasma protein profiling by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry. *Fertil. Steril.* **87**, 988–990 (2007).
424. Long, X., Jiang, P., Zhou, L. & Zhang, W. Evaluation of novel serum biomarkers and the proteomic differences of endometriosis and adenomyosis using MALDI-TOF-MS. *Arch. Gynecol. Obstet.* **288**, 201–205 (2013).
425. Jing, J. *et al.* Two novel serum biomarkers for endometriosis screened by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry and their change after laparoscopic removal of endometriosis. *Fertil. Steril.* **92**, 1221–1227 (2009).
426. Nabeta, M. *et al.* Serum anti-PDIK1L autoantibody as a novel marker for endometriosis. *Fertil. Steril.* **94**, 2552-2557.e1 (2010).
427. Nabeta, M. *et al.* Identification of anti-α-enolase autoantibody as a novel serum marker for endometriosis. *Proteomics - Clin. Appl.* **3**, 1201–1210 (2009).
428. Nabeta, M., Abe, Y., Takaoka, Y., Kusanagi, Y. & Ito, M. Identification of anti-syntaxin 5 autoantibody as a novel serum marker of endometriosis. *J. Reprod. Immunol.* **91**, 48–55 (2011).
429. Ghazi, N. *et al.* 1H NMR- based metabolomics approaches as non-invasive tools for diagnosis of endometriosis. *Int. J. Reprod. Biomed.* **14**, 1–8 (2016).
430. Vouk, K. *et al.* Discovery of phosphatidylcholines and sphingomyelins as biomarkers for ovarian endometriosis. *Hum. Reprod.* **27**, 2955–2965 (2012).
431. Dutta, M. *et al.* A metabonomics approach as a means for identification of potential biomarkers for early diagnosis of endometriosis. *Mol. Biosyst.* **8**, 3281–3287 (2012).
432. Jana, S. K. *et al.* 1H NMR based targeted metabolite profiling for understanding the complex relationship connecting oxidative stress with endometriosis. *Biomed Res. Int.* **2013**, (2013).
433. Cirulli, E. T. & Goldstein, D. B. Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nature Reviews Genetics* vol. 11 415–425 (2010).
434. Koboldt, D. C., Steinberg, K. M., Larson, D. E., Wilson, R. K. & Mardis, E. R. XThe next-generation sequencing revolution and its impact on genomics. *Cell* vol. 155 27 (2013).
435. Voight, B. F. *et al.* The Metabochip, a Custom Genotyping Array for Genetic Studies of Metabolic, Cardiovascular, and Anthropometric Traits. *PLoS Genet.* **8**, (2012).
436. Ragoussis, J. Genotyping technologies for genetic research. *Annu. Rev. Genomics Hum. Genet.* **10**, 117–133 (2009).
437. Hirschhorn, J. N. & Daly, M. J. Genome-wide association studies for common diseases and complex traits. *Nature Reviews Genetics* vol. 6 95–108 (2005).
438. Wang, W. Y. S., Barratt, B. J., Clayton, D. G. & Todd, J. A. Genome-wide association studies: Theoretical and practical concerns. *Nature Reviews Genetics* vol. 6 109–118 (2005).

439. Ng, S. B. *et al.* Targeted capture and massively parallel sequencing of 12 human exomes. *Nature* **461**, 272–276 (2009).
440. Rahmioglu, N., Montgomery, G. W. & Zondervan, K. T. Genetics of endometriosis. *Women's Heal.* **11**, 577–586 (2015).
441. Vassilopoulou, L. *et al.* Defining the genetic profile of endometriosis (Review). *Exp. Ther. Med.* **17**, 3267–3281 (2019).
442. Kobayashi, H., Imanaka, S., Nakamura, H. & Tsuji, A. Understanding the role of epigenomic, genomic and genetic alterations in the development of endometriosis (review). *Molecular Medicine Reports* vol. 9 1483–1505 (2014).
443. Sapkota, Y. *et al.* Meta-analysis identifies five novel loci associated with endometriosis highlighting key genes involved in hormone metabolism. *Nat. Commun.* **8**, 1–12 (2017).
444. Uimari, O. *et al.* Genome-wide genetic analyses highlight mitogen-activated protein kinase (MAPK) signaling in the pathogenesis of endometriosis. *Hum. Reprod.* **32**, 780–793 (2017).
445. Rahmioglu, N. *et al.* Variability of genome-wide DNA methylation and mRNA expression profiles in reproductive and endocrine disease related tissues. *Epigenetics* **12**, 897–908 (2017).
446. Piunti, A. & Shilatifard, A. Epigenetic balance of gene expression by polycomb and compass families. *Science (80-.).* **352**, aad9780 (2016).
447. Taudt, A., Colomé-Tatché, M. & Johannes, F. Genetic sources of population epigenomic variation. *Nature Reviews Genetics* vol. 17 319–332 (2016).
448. Liu, L., Li, Y. & Tollefsbol, T. O. Gene-environment interactions and epigenetic basis of human diseases. *Current Issues in Molecular Biology* vol. 10 25–36 (2008).
449. Gut, P. & Verdin, E. The nexus of chromatin regulation and intermediary metabolism. *Nature* vol. 502 489–498 (2013).
450. Zhu, J. *et al.* Genome-wide chromatin state transitions associated with developmental and environmental cues. *Cell* **152**, 642–654 (2013).
451. Nestler, E. J. Transgenerational Epigenetic Contributions to Stress Responses: Fact or Fiction? *PLoS Biol.* **14**, (2016).
452. Barrès, R. & Zierath, J. R. The role of diet and exercise in the transgenerational epigenetic landscape of T2DM. *Nature Reviews Endocrinology* vol. 12 441–451 (2016).
453. Horvath, S. DNA methylation age of human tissues and cell types. *Genome Biol.* **14**, R115 (2013).
454. Kim, M. *et al.* DNA methylation as a biomarker for cardiovascular disease risk. *PLoS One* **5**, e9692 (2010).
455. Baylin, S. B. *et al. Abberant patterns of DNA methylation, chromatin formation and gene expression in cancer*. *Human Molecular Genetics* vol. 10 (2001).
456. Multhaup, M. L. *et al.* Mouse-human experimental epigenetic analysis unmasks dietary targets and genetic liability for diabetic phenotypes. *Cell Metab.* **21**, 138–149 (2015).
457. Raghuraman, S., Donkin, I., Versteyhe, S., Barrès, R. & Simar, D. The Emerging Role of Epigenetics in Inflammation and Immunometabolism. *Trends in Endocrinology and Metabolism* vol. 27 782–795 (2016).
458. Zelenko, Z., Aghajanova, L., Irwin, J. C. & Giudice, L. C. Nuclear receptor, coregulator signaling, and chromatin remodeling pathways suggest involvement of the epigenome in the steroid hormone response of endometrium and abnormalities in endometriosis. *Reprod. Sci.* **19**, 152–162 (2012).
459. Izawa, M., Taniguchi, F., Terakawa, N. & Harada, T. Epigenetic aberration of gene expression in endometriosis. *Front. Biosci. - Elit.* **5 E**, 900–910 (2013).
460. Goulielmos, G. N. *et al.* Endometriosis research in the -omics era. *Gene* vol. 741 144545 (2020).
461. Xue, Q. *et al.* Transcriptional activation of steroidogenic factor-1 by hypomethylation of the 5′ CpG island in endometriosis. *J. Clin. Endocrinol. Metab.* **92**, 3261–3267 (2007).
462. Bulun, S. E. *et al.* Molecular biology of endometriosis: From aromatase to genomic abnormalities. *Semin. Reprod. Med.* **33**, 220–224 (2015).
463. Xue, Q. *et al.* Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. *Biol. Reprod.* **77**, 681–687 (2007).

464. Monsivais, D. *et al.* ERβ- and prostaglandin E2-regulated pathways integrate cell proliferation via Ras-like and estrogen-regulated growth inhibitor in endometriosis. *Mol. Endocrinol.* **28**, 1304–1315 (2014).
465. Bulun, S. E. *et al.* Endometriosis. *Endocr. Rev.* **40**, 1048–1079 (2019).
466. Zhao, W. *et al.* Aberrant methylation of the IL-12B promotor region contributes to the risk of developing ovarian endometriosis. *Mol. Reprod. Dev.* **86**, 632–638 (2019).
467. Dunham, I. *et al.* An integrated encyclopedia of DNA elements in the human genome. *Nature* **489**, 57–74 (2012).
468. Trapnell, C. *et al.* Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat. Biotechnol.* **28**, 511–515 (2010).
469. Arnes, L., Akerman, I., Balderes, D. A., Ferrer, J. & Sussel, L. βlinc1 encodes a long noncoding RNA that regulates islet β-cell formation and function. *Genes Dev.* **30**, 502–507 (2016).
470. Morán, I. *et al.* Human β cell transcriptome analysis uncovers lncRNAs that are tissue-specific, dynamically regulated, and abnormally expressed in type 2 diabetes. *Cell Metab.* **16**, 435–448 (2012).
471. Ishii, N. *et al.* Identification of a novel non-coding RNA, MIAT, that confers risk of myocardial infarction. *J. Hum. Genet.* **51**, 1087–1099 (2006).
472. Gupta, R. A. *et al.* Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature* **464**, 1071–1076 (2010).
473. Schmitz, S. U., Grote, P. & Herrmann, B. G. Mechanisms of long noncoding RNA function in development and disease. *Cellular and Molecular Life Sciences* vol. 73 2491–2509 (2016).
474. Giudice, L. C., Talbi, S., Hamilton, A. & Lessey, B. A. Transcriptomics. in *The Endometrium: Molecular, Cellular and Clinical Perspectives, Second Edition* 193–222 (CRC Press, 2008). doi:10.2174/138920008783884759.
475. Monsivais, D. *et al.* Activated glucocorticoid and eicosanoid pathways in endometriosis. *Fertil. Steril.* **98**, 117–125 (2012).
476. James, P. Protein identification in the post-genome era: The rapid rise of proteomics. *Q. Rev. Biophys.* **30**, 279–331 (1997).
477. Hein, M. Y., Sharma, K., Cox, J. & Mann, M. Proteomic Analysis of Cellular Systems. in *Handbook of Systems Biology* 3–25 (Elsevier Inc., 2013). doi:10.1016/B978-0-12-385944-0.00001-0.
478. Selevsek, N. *et al.* Reproducible and consistent quantification of the saccharomyces cerevisiae proteome by SWATH-mass spectrometry. *Mol. Cell. Proteomics* **14**, 739–749 (2015).
479. Beck, H. C. *et al.* Quantitative proteomic analysis of post-translational modifications of human histones. *Mol. Cell. Proteomics* **5**, 1314–1325 (2006).
480. Mann, M. & Jensen, O. N. Proteomic analysis of post-translational modifications. *Nature Biotechnology* vol. 21 255–261 (2003).
481. Wu, R. *et al.* A large-scale method to measure absolute protein phosphorylation stoichiometries. *Nat. Methods* **8**, 677–683 (2011).
482. Choudhary, C. & Mann, M. Decoding signalling networks by mass spectrometry-based proteomics. *Nature Reviews Molecular Cell Biology* vol. 11 427–439 (2010).
483. Domon, B. & Aebersold, R. Mass spectrometry and protein analysis. *Science* vol. 312 212–217 (2006).
484. Yates, J. R., Eng, J. K., McCormack, A. L. & Schieltz, D. Method to Correlate Tandem Mass Spectra of Modified Peptides to Amino Acid Sequences in the Protein Database. *Anal. Chem.* **67**, 1426–1436 (1995).
485. Siristatidis, C. S. What have the 'omics done for endometriosis? *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.* **15**, RA116-23 (2009).
486. May, K. E., Villar, J., Kirtley, S., Kennedy, S. H. & Becker, C. M. Endometrial alterations in endometriosis: A systematic review of putative biomarkers. *Hum. Reprod. Update* **17**, 637–653 (2011).

487. Wang, L., Liu, H. Y., Shi, H. H., Lang, J. H. & Sun, W. Urine peptide patterns for non-invasive diagnosis of endometriosis: A preliminary prospective study. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **177**, 23–28 (2014).
488. Irungu, S. *et al.* Discovery of non-invasive biomarkers for the diagnosis of endometriosis. *Clin. Proteomics* **16**, 14 (2019).
489. Ab, S., Srivastava, P. & Shivaji, S. Understanding the pathogenesis of endometriosis through proteomics: Recent advances and future prospects. *Proteomics - Clin. Appl.* **8**, 86–98 (2014).
490. Nicholson, J. K. & Wilson, I. D. Understanding 'global' systems biology: Metabonomics and the continuum of metabolism. *Nature Reviews Drug Discovery* vol. 2 668–676 (2003).
491. Ghazalpour, A. *et al.* Genetic regulation of mouse liver metabolite levels. *Mol. Syst. Biol.* **10**, 730 (2014).
492. Shin, S. Y. *et al.* An atlas of genetic influences on human blood metabolites. *Nat. Genet.* **46**, 543–550 (2014).
493. Kettunen, J. *et al.* Genome-wide association study identifies multiple loci influencing human serum metabolite levels. *Nat. Genet.* **44**, 269–276 (2012).
494. Gieger, C. *et al.* Genetics meets metabolomics: A genome-wide association study of metabolite profiles in human serum. *PLoS Genet.* **4**, (2008).
495. Madsen, R., Lundstedt, T. & Trygg, J. Chemometrics in metabolomics-A review in human disease diagnosis. *Anal. Chim. Acta* **659**, 23–33 (2010).
496. Serkova, N. J., Spratlin, J. L. & Eckhardt, S. G. NMR-based metabolomics: Translational application and treatment of cancer. *Current Opinion in Molecular Therapeutics* vol. 9 572–585 (2007).
497. Samuel, J. L. *et al.* Genomics in cardiac metabolism. *Cardiovasc. Res.* **79**, 218–227 (2008).
498. Sébédio, J. L., Pujos-Guillot, E. & Ferrara, M. Metabolomics in evaluation of glucose disorders. *Current Opinion in Clinical Nutrition and Metabolic Care* vol. 12 412–418 (2009).
499. Watkins, S. M., Reifsnyder, P. R., Pan, H. J., Bruce German, J. & Leiter, E. H. Lipid metabolome-wide effects of the PPARγ agonist rosiglitazone. *J. Lipid Res.* **43**, 1809–1817 (2002).
500. Prieto, L. *et al.* Analysis of follicular fluid and serum markers of oxidative stress in women with infertility related to endometriosis. *Fertil. Steril.* **98**, 126–130 (2012).
501. Dutta, M. *et al.* Author Correction: Metabolomics reveals perturbations in endometrium and serum of minimal and mild endometriosis (Scientific Reports, (2018), 8, 1, (6466), 10.1038/s41598-018-23954-7). *Scientific Reports* vol. 10 1–1 (2020).
502. Cordeiro, F. B. *et al.* Lipidomics analysis of follicular fluid by ESI-MS reveals potential biomarkers for ovarian endometriosis. *J. Assist. Reprod. Genet.* **32**, 1817–1825 (2015).
503. Domínguez, F. *et al.* Lipidomic profiling of endometrial fluid in women with ovarian endometriosis. *Biol. Reprod.* **96**, 772–779 (2017).
504. Lee, Y. H. *et al.* Limited value of pro-inflammatory oxylipins and cytokines as circulating biomarkers in endometriosis - A targeted 'omics study. *Sci. Rep.* **6**, 1–7 (2016).
505. Wenk, M. R. The emerging field of lipidomics. *Nature Reviews Drug Discovery* vol. 4 594–610 (2005).
506. Watson, A. D. Thematic review series: Systems biology approaches to metabolic and cardiovascular disorders. Lipidomics: A global approach to lipid analysis in biological systems. *Journal of Lipid Research* vol. 47 2101–2111 (2006).
507. Han, X. Neurolipidomics: Challenges and developments. *Frontiers in Bioscience* vol. 12 2601–2615 (2007).
508. Santulli, P. *et al.* Sphingosine pathway deregulation in endometriotic tissues. *Fertil. Steril.* **97**, (2012).
509. Org, E. *et al.* Genetic and environmental control of host-gut microbiota interactions. *Genome Res.* **25**, 1558–1569 (2015).
510. Huttenhower, C. *et al.* Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214 (2012).

511. Petersen, C. & Round, J. L. Defining dysbiosis and its influence on host immunity and disease. *Cellular Microbiology* vol. 16 1024–1033 (2014).
512. Trompette, A. *et al.* Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med.* **20**, 159–166 (2014).
513. Clemente, J. C., Ursell, L. K., Parfrey, L. W. & Knight, R. The impact of the gut microbiota on human health: An integrative view. *Cell* vol. 148 1258–1270 (2012).
514. Laschke, M. W. & Menger, M. D. The gut microbiota: A puppet master in the pathogenesis of endometriosis? *Am. J. Obstet. Gynecol.* **215**, 68.e1-68.e4 (2016).
515. Chen, C. *et al.* The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. *Nat. Commun.* **8**, 1–11 (2017).
516. Ravel, J. *et al.* Vaginal microbiome of reproductive-age women. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 4680–4687 (2011).
517. Gajer, P. *et al.* Temporal dynamics of the human vaginal microbiota. *Sci. Transl. Med.* **4**, 132ra52-132ra52 (2012).
518. Cregger, M. A. *et al.* Reproductive Microbiomes: Using the Microbiome as a Novel Diagnostic Tool for Endometriosis. *Reprod. Immunol. Open Access* **02**, (2017).
519. Verstraelen, H. *et al.* Characterisation of the human uterine microbiome in non-pregnant women through deep sequencing of the V1-2 region of the 16S rRNA gene. *PeerJ* **2016**, e1602 (2016).
520. Puca, J. & F. Hoyne, G. Microbial dysbiosis and disease pathogenesis of endometriosis, could there be a link? *Allied J. Med. Res.* **01**, (2017).
521. Flores, R. *et al.* Fecal microbial determinants of fecal and systemic estrogens and estrogen metabolites: A cross-sectional study. *J. Transl. Med.* **10**, 253 (2012).
522. Chadchan, S. B. *et al.* Antibiotic therapy with metronidazole reduces endometriosis disease progression in mice: A potential role for gut microbiota. *Hum. Reprod.* **34**, 1106–1116 (2019).
523. Xu, J. *et al.* Dysbiosis of gut microbiota contributes to chronic stress in endometriosis patients via activating inflammatory pathway. *Reprod. Dev. Med.* **1**, 221–227 (2017).
524. Missmer, S. A. *et al.* A prospective study of dietary fat consumption and endometriosis risk. *Hum. Reprod.* **25**, 1528–1535 (2010).
525. Shapiro, S. C. *Encyclopedia of Artificial Intelligence*. *Encyclopedia of Artificial Intelligence* (John Wiley & Sons, Inc., 2011). doi:10.4018/978-1-59904-849-9.
526. McCorduck, P. & Cfe, C. *Machines Who Think*. *Machines Who Think* (2004) doi:10.1201/9780429258985.
527. Ramesh, A. N., Kambhampati, C., Monson, J. R. T. & Drew, P. J. Artificial intelligence in medicine. *Annals of the Royal College of Surgeons of England* vol. 86 334–338 (2004).
528. Turing, A. M. *Computing machinery and intelligence*. *Machine Intelligence: Perspectives on the Computational Model* vol. 59 (2012).
529. Hamet, P. & Tremblay, J. Artificial intelligence in medicine. *Metabolism.* **69**, S36–S40 (2017).
530. Pennachin, C. & Goertzel, B. Contemporary Approaches to Artificial General Intelligence. *Cogn. Technol.* **8**, 1–30 (2007).
531. de Garis, H. Artificial Brains. *Cogn. Technol.* **8**, 159–174 (2007).
532. Kolker, E., Özdemir, V. & Kolker, E. How Healthcare Can Refocus on Its Super-Customers (Patients, n =1) and Customers (Doctors and Nurses) by Leveraging Lessons from Amazon, Uber, and Watson. *Omi. A J. Integr. Biol.* **20**, 329–333 (2016).
533. Dilsizian, S. E. & Siegel, E. L. Artificial intelligence in medicine and cardiac imaging: Harnessing big data and advanced computing to provide personalized medical diagnosis and treatment. *Curr. Cardiol. Rep.* **16**, 1–8 (2014).
534. Hajirasouliha, I. & Elemento, O. Precision medicine and artificial intelligence: overview and relevance to reproductive medicine. *Fertility and Sterility* vol. 114 908–913 (2020).
535. Pre-Processing from the Data Set.
536. Bermingham, M. L. *et al.* Application of high-dimensional feature selection: Evaluation for genomic prediction in man. *Sci. Rep.* **5**, (2015).

537. Theofilatos, K. *et al.* Predicting protein complexes from weighted protein-protein interaction graphs with a novel unsupervised methodology: Evolutionary enhanced Markov clustering. *Artif. Intell. Med.* **63**, 181–189 (2015).
538. Rapakoulia, T. *et al.* EnsembleGASVR: A novel ensemble method for classifying missense single nucleotide polymorphisms. *Bioinformatics* **30**, 2324–2333 (2014).
539. Poplin, R. *et al.* A universal SNP and small-indel variant caller using deep neural networks. *Nat. Biotechnol.* **36**, 983–987 (2018).
540. Zhou, J. *et al.* Whole-genome deep-learning analysis identifies contribution of noncoding mutations to autism risk. *Nat. Genet.* **51**, 973–980 (2019).
541. Ainscough, B. J. *et al.* A deep learning approach to automate refinement of somatic variant calling from cancer sequencing data. *Nat. Genet.* **50**, 1735–1743 (2018).
542. Bhinder, B. & Elemento, O. Towards a better cancer precision medicine: Systems biology meets immunotherapy. *Current Opinion in Systems Biology* vol. 2 67–73 (2017).
543. Adam, G. *et al.* Machine learning approaches to drug response prediction: challenges and recent progress. *npj Precis. Oncol.* **4**, 19 (2020).
544. Esteva, A. *et al.* Dermatologist-level classification of skin cancer with deep neural networks. *Nature* **542**, 115–118 (2017).
545. Gulshan, V. *et al.* Development and validation of a deep learning algorithm for detection of diabetic retinopathy in retinal fundus photographs. *JAMA - J. Am. Med. Assoc.* **316**, 2402–2410 (2016).
546. De Fauw, J. *et al.* Clinically applicable deep learning for diagnosis and referral in retinal disease. *Nat. Med.* **24**, 1342–1350 (2018).
547. Coudray, N. *et al.* Classification and mutation prediction from non–small cell lung cancer histopathology images using deep learning. *Nat. Med.* **24**, 1559–1567 (2018).
548. Lakhani, P. & Sundaram, B. Deep learning at chest radiography: Automated classification of pulmonary tuberculosis by using convolutional neural networks. *Radiology* **284**, 574–582 (2017).
549. Ardila, D. *et al.* End-to-end lung cancer screening with three-dimensional deep learning on low-dose chest computed tomography. *Nat. Med.* **25**, 954–961 (2019).
550. Khosravi, P., Kazemi, E., Imielinski, M., Elemento, O. & Hajirasouliha, I. Deep Convolutional Neural Networks Enable Discrimination of Heterogeneous Digital Pathology Images. *EBioMedicine* **27**, 317–328 (2018).
551. Byrne, M. F. *et al.* Real-time differentiation of adenomatous and hyperplastic diminutive colorectal polyps during analysis of unaltered videos of standard colonoscopy using a deep learning model. *Gut* **68**, 94–100 (2019).
552. Cornet, G. Robot companions and ethiCs a pragmatiC appRoaCh of ethiCal dEsign. *J. Int. Bioethique* **33**, 49–58 (2013).
553. Larson, J. A., Johnson, M. H. & Bhayani, S. B. Application of surgical safety standards to robotic surgery: Five principles of ethics for nonmaleficence. *J. Am. Coll. Surg.* **218**, 290–293 (2014).
554. Simonov, M. & Delconte, G. Humanoid assessing rehabilitative exercises. *Methods Inf. Med.* **54**, 114–121 (2015).
555. Brooks, D. & Howard, A. M. A computational method for physical rehabilitation assessment. in *2010 3rd IEEE RAS and EMBS International Conference on Biomedical Robotics and Biomechatronics, BioRob 2010* 442–447 (IEEE, 2010). doi:10.1109/BIOROB.2010.5626047.
556. Lee, C. S., Nagy, P. G., Weaver, S. J. & Newman-Toker, D. E. Cognitive and system factors contributing to diagnostic errors in radiology. *American Journal of Roentgenology* vol. 201 611–617 (2013).
557. Winters, B. *et al.* Diagnostic errors in the intensive care unit: A systematic review of autopsy studies. *BMJ Quality and Safety* vol. 21 894–902 (2012).
558. Graber, M. L., Franklin, N. & Gordon, R. Diagnostic error in internal medicine. *Arch. Intern. Med.* **165**, 1493–1499 (2005).

559. Weingart, S. N., Wilson, R. M. L., Gibberd, R. W. & Harrison, B. Epidemiology of medical error. *British Medical Journal* vol. 320 774–777 (2000).
560. Jha, S. & Topol, E. J. Adapting to artificial intelligence: Radiologists and pathologists as information specialists. *JAMA - Journal of the American Medical Association* vol. 316 2353–2354 (2016).
561. Patel, V. L. *et al.* The coming of age of artificial intelligence in medicine. *Artif. Intell. Med.* **46**, 5–17 (2009).
562. Makary, M. A. & Daniel, M. Medical error-the third leading cause of death in the US. *BMJ* **353**, (2016).
563. Abhinav, G. V. K. S. & Naga Subrahmanyam, S. *Artificial Intelligence in Healthcare*. *Journal of Drug Delivery and Therapeutics* vol. 9 (2019).
564. Yoldemir, T. Artificial intelligence and women's health. *Climacteric* vol. 23 1–2 (2020).
565. Steimann, F. On the use and usefulness of fuzzy sets in medical AI. *Artif. Intell. Med.* **21**, 131–137 (2001).
566. Rosenblatt, F. The perceptron: A probabilistic model for information storage and organization in the brain. *Psychol. Rev.* **65**, 386–408 (1958).
567. Boon, M. E., Kok, L. P. & Under, J. Neural network processing can provide means to catch errors that slip through human screening of pap smears. *Diagn. Cytopathol.* **9**, 411–416 (1993).
568. Solomon, H. M. & Frist, S. PAPNET testing for HSILs: The few cell/small cell challenge. *Acta Cytol.* **42**, 253–259 (1998).
569. Downs, J., Harrison, R. F., Kennedy, R. L. & Cross, S. S. Application of the fuzzy ARTMAP neural network model to medical pattern classification tasks. *Artif. Intell. Med.* **8**, 403–428 (1996).
570. Ashizawa, K. *et al.* Artificial neural networks in chest radiography: Application to the differential diagnosis of interstitial lung disease. *Acad. Radiol.* **6**, 2–9 (1999).
571. Tailor, A., Jurkovic, D., Bourne, T. H., Collins, W. P. & Campbell, S. Sonographic prediction of malignancy in adnexal masses using an artificial neural network. *BJOG An Int. J. Obstet. Gynaecol.* **106**, 21–30 (1999).
572. Matsuki, Y. *et al.* Usefulness of an artificial neural network for differentiating benign from malignant pulmonary nodules on high-resolution CT: Evaluation with receiver operating characteristic analysis. *Am. J. Roentgenol.* **178**, 657–663 (2002).
573. Lucht, R., Delorme, S. & Brix, G. Neural network-based segmentation of dynamic MR mammographic images. *Magn. Reson. Imaging* **20**, 147–154 (2002).
574. Fisher, R. E., Scott, J. A. & Palmer, E. L. Neural networks in ventilation-perfusion imaging: Part I. Effects of interpretive criteria and network architecture. *Radiology* **198**, 699–706 (1996).
575. Hedén, B., Edenbrandt, L., Haisty, W. K. & Pahlm, O. Artificial neural networks for the electrocardiographic diagnosis of healed myocardial infarction. *Am. J. Cardiol.* **74**, 5–8 (1994).
576. Yang, T. F., Devine, B. & Macfarlane, P. W. Artificial neural networks for the diagnosis of atrial fibrillation. *Med. Biol. Eng. Comput.* **32**, 615–619 (1994).
577. Dassen, W. R. M. *et al.* Evaluation of new self-learning techniques for the generation of criteria for differentiation of wide-QRS tachycardia in supraventricular tachycardia and ventricular tachycardia. *Clin. Cardiol.* **18**, 103–108 (1995).
578. Walczak, S. & Nowack, W. J. An Artificial Neural Network Approach to Diagnosing Epilepsy Using Lateralized Bursts of Theta EEGs. *J. Med. Syst.* **25**, 9–20 (2001).
579. Schaltenbrand, N. *et al. Sleep stage scoring using the neural network model: Comparison between visual and automatic analysis in normal subjects and patients*. *Sleep* vol. 19 https://academic.oup.com/sleep/article-abstract/19/1/26/2749751 (1996).
580. Abel, E. W., Zacharia, P. C., Forster, A. & Farrow, T. L. Neural network analysis of the EMG interference pattern. *Med. Eng. Phys.* **18**, 12–17 (1996).
581. Smith, J. H., Graham, J. & Taylor, R. J. The application of an artificial neural network to Doppler ultrasound waveforms for the classification of arterial disease. *Int. J. Clin. Monit. Comput.* **13**, 85–91 (1996).

582. Spencer, R. G., Lessard, C. S., Davila, F. & Etter, B. Self-organising discovery, recognition and prediction of haemodynamic patterns in the intensive care unit. *Med. Biol. Eng. Comput.* **35**, 117–123 (1997).
583. Karakitsos, P. *et al.* Potential of the back propagation neural network in the discrimination of benign from malignant gastric cells. *Anal. Quant. Cytol. Histol.* **18**, 245–250 (1996).
584. Karakitsos, P., Cochand-Priollet, B., Guillausseau, P. J. & Pouliakis, A. Potential of the back propagation neural network in the morphologic examination of thyroid lesions. *Anal. Quant. Cytol. Histol.* **18**, 494–500 (1996).
585. Brickley, M. R., Cowpe, J. G. & Shepherd, J. P. Performance of a computer simulated neural network trained to categorise normal, premalignant and malignant oral smears. *J. Oral Pathol. Med.* **25**, 424–428 (1996).
586. Hurst, R. E., Bonner, R. B., Ashenayi, K., Veltri, R. W. & Hemstreet, G. P. 3rd. Neural net-based identification of cells expressing the p300 tumor-related antigen using fluorescence image analysis. *Cytometry* **27**, 36–42 (1997).
587. Truong, H., Morimoto, R., Walts, A. E., Erler, B. & Marchevsky, A. Neural networks as an aid in the diagnosis of lymphocyte-rich effusions. *Anal. Quant. Cytol. Histol.* **17**, 48–54 (1995).
588. Vogiatzi, P., Pouliakis, A. & Siristatidis, C. An artificial neural network for the prediction of assisted reproduction outcome. *J. Assist. Reprod. Genet.* **36**, 1441–1448 (2019).
589. VerMilyea, M. *et al.* Development of an artificial intelligence-based assessment model for prediction of embryo viability using static images captured by optical light microscopy during IVF. *Hum. Reprod.* **35**, 770–784 (2020).
590. Zaninovic, N. & Rosenwaks, Z. Artificial intelligence in human in vitro fertilization and embryology. *Fertility and Sterility* vol. 114 914–920 (2020).
591. Khosravi, P. *et al.* Deep learning enables robust assessment and selection of human blastocysts after in vitro fertilization. *npj Digit. Med.* **2**, 21 (2019).
592. Kragh, M. F., Rimestad, J., Berntsen, J. & Karstoft, H. Automatic grading of human blastocysts from time-lapse imaging. *Comput. Biol. Med.* **115**, 103494 (2019).
593. Fedele, L. *et al.* Evaluation of a new questionnaire for the presurgical diagnosis of bladder endometriosis. *Hum. Reprod.* **22**, 2698–2701 (2007).
594. Eskenazi, B. *et al.* Validation study of nonsurgical diagnosis of endometriosis. *Fertil. Steril.* **76**, 929–935 (2001).
595. Chapron, C. *et al.* Presurgical diagnosis of posterior deep infiltrating endometriosis based on a standardized questionnaire. *Hum. Reprod.* **20**, 507–513 (2005).
596. Fassbender, A. *et al.* World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonisation Project: IV. Tissue collection, processing, and storage in endometriosis research. *Fertility and Sterility* vol. 102 1244–1253 (2014).
597. Vitonis, A. F. *et al.* World Endometriosis Research Foundation Endometriosis Phenome and biobanking harmonization project: II. Clinical and covariate phenotype data collection in endometriosis research. *Fertility and Sterility* vol. 102 1223–1232 (2014).
598. Johnson, N. P. & Miller, L. M. EPHect - the Endometriosis Phenome (and Biobanking) Harmonisation Project - may be very helpful for clinicians and the women they are treating. *F1000Research* **6**, 14 (2017).
599. Harwood, D. T. & Handelsman, D. J. Development and validation of a sensitive liquid chromatography-tandem mass spectrometry assay to simultaneously measure androgens and estrogens in serum without derivatization. *Clin. Chim. Acta* **409**, 78–84 (2009).
600. Häkkinen, M. R. *et al.* Analysis by LC–MS/MS of endogenous steroids from human serum, plasma, endometrium and endometriotic tissue. *J. Pharm. Biomed. Anal.* **152**, 165–172 (2018).
601. Dunning, M. J., Smith, M. L., Ritchie, M. E. & Tavaré, S. Beadarray: R classes and methods for Illumina bead-based data. *Bioinformatics* **23**, 2183–2184 (2007).
602. Gabriel, M. *et al.* A relational database to identify differentially expressed genes in the endometrium and endometriosis lesions. *Sci. Data* **7**, 284 (2020).

603. Gentleman, R. C. *et al.* Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.* **5**, R80 (2004).
604. Datta, A. & Rawat, G. S. Foraging Patterns of Sympatric Hornbills during the Nonbreeding Season in Arunachal Pradesh, Northeast India. *Biotropica* **35**, 208–218 (2003).
605. Müller, C. *et al.* Removing batch effects from longitudinal gene expression - Quantile normalization plus comBat as best approach for microarray transcriptome data. *PLoS One* **11**, e0156594 (2016).
606. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.* **215**, 403–410 (1990).
607. Allen, J. D. *et al.* Probe mapping across multiple microarray platforms. *Brief. Bioinform.* **13**, 547–554 (2012).
608. bioDBnet - Biological Database Network. http://biodbnet.abcc.ncifcrf.gov/ (2015).
609. Index of /pub/databases/genenames/new/tsv/. ftp://ftp.ebi.ac.uk/pub/databases/genenames/new/tsv/.
610. Durinck, S., Spellman, P. T., Birney, E. & Huber, W. Mapping identifiers for the integration of genomic datasets with the R/ Bioconductor package biomaRt. *Nat. Protoc.* **4**, 1184–1191 (2009).
611. Smedley, D. *et al.* The BioMart community portal: An innovative alternative to large, centralized data repositories. *Nucleic Acids Res.* **43**, W589–W598 (2015).
612. Johnson, W. E., Li, C. & Rabinovic, A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* **8**, 118–127 (2007).
613. Leek, J. T., Johnson, W. E., Parker, H. S., Jaffe, A. E. & Storey, J. D. The SVA package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* **28**, 882–883 (2012).
614. Ritchie, M. E. *et al.* Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* **43**, e47 (2015).
615. limma package | R Documentation. https://www.rdocumentation.org/packages/limma/versions/3.28.14.
616. Niehrs, C. The complex world of WNT receptor signalling. *Nature Reviews Molecular Cell Biology* vol. 13 767–779 (2012).
617. Clevers, H., Loh, K. M. & Nusse, R. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. *Science* vol. 346 (2014).
618. Green, J., Nusse, R. & van Amerongen, R. The role of Ryk and Ror receptor tyrosine kinases in wnt signal transduction. *Cold Spring Harb. Perspect. Biol.* **6**, (2014).
619. Nusse, R. The Wnt Homepage. *Stanford University* http://www.stanford.edu/group/nusselab/cgi-bin/wnt/ (2016).
620. Herbst, A. *et al.* Comprehensive analysis of β-catenin target genes in colorectal carcinoma cell lines with deregulated Wnt/β-catenin signaling. *BMC Genomics* **15**, (2014).
621. Messeguer, X. *et al. PROMO: Detection of known transcription regulatory elements using species-tailored searches*. *Bioinformatics* vol. 18 http://www.lsi.upc.es/~alggen/recerca/promo/figuraBioinformatics.html. (2002).
622. Team., R. C. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *https://www.R-project.org/* (2019).
623. Villanueva, R. A. M. & Chen, Z. J. *ggplot2: Elegant Graphics for Data Analysis (2nd ed.)*. *Measurement: Interdisciplinary Research and Perspectives* vol. 17 (Springer-Verlag New York, 2019).
624. Van Rossum, G. & Drake, F. L. Python Tutorial. *Python Software Foundation* 1–136 (2012).
625. Kohavi, R. & John, G. H. Wrappers for feature subset selection. *Artif. Intell.* **97**, 273–324 (1997).
626. Guyon, I., Weston, J., Barnhill, S. & Vapnik, V. Gene selection for cancer classification using support vector machines. *Mach. Learn.* **46**, 389–422 (2002).
627. Breiman, L. Random forests. *Mach. Learn.* **45**, 5–32 (2001).
628. Pedregosa, F. \emphet al. *et al.* Scikit-learn: Machine Learning in {P}ython. *J. Mach. Learn. Res.* **12**, 2825–2830 (2011).

629. Han, J., Kamber, M. & Pei, J. *Data Mining : Concepts and Techniques : Concepts and Techniques (3rd Edition)*. *Data Mining* (Morgan Kaufmann, 2012).
630. Luo, S. T. & Cheng, B. W. Diagnosing breast masses in digital mammography using feature selection and ensemble methods. *J. Med. Syst.* **36**, 569–577 (2012).
631. Breiman, L. Bagging predictors. *Mach. Learn.* **24**, 123–140 (1996).
632. Golland, P., Liang, F., Mukherjee, S. & Panchenko, D. Permutation tests for classification. in *Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)* vol. 3559 LNAI 501–515 (2005).
633. Varma, S. & Simon, R. Bias in error estimation when using cross-validation for model selection. *BMC Bioinformatics* **7**, 91 (2006).
634. Heinosalo, T. *et al.* Secreted frizzled-related protein 2 (SFRP2) expression promotes lesion proliferation via canonical WNT signaling and indicates lesion borders in extraovarian endometriosis. *Hum. Reprod.* **33**, 817–831 (2018).
635. Buechling, T. & Boutros, M. Wnt Signaling. Signaling at and Above the Receptor Level. in *Current Topics in Developmental Biology* vol. 97 21–53 (Academic Press Inc., 2011).
636. Matsuzaki, S., Darcha, C., Maleysson, E., Canis, M. & Mage, G. Impaired down-regulation of E-cadherin and β-catenin protein expression in endometrial epithelial cells in the mid-secretory endometrium of infertile patients with endometriosis. *J. Clin. Endocrinol. Metab.* **95**, 3437–3445 (2010).
637. Matsuzaki, S. & Darcha, C. Epithelial to mesenchymal transition-like and mesenchymal to epithelial transition-like processes might be involved in the pathogenesis of pelvic endometriosis. *Hum. Reprod.* **27**, 712–721 (2012).
638. Matsuzaki, S. & Darcha, C. In Vitro Effects of a Small-Molecule Antagonist of the Tcf/ß-Catenin Complex on Endometrial and Endometriotic Cells of Patients with Endometriosis. *PLoS One* **8**, e61690 (2013).
639. Matsuzaki, S. & Darcha, C. Involvement of the Wnt/β-Catenin Signaling Pathway in the Cellular and Molecular Mechanisms of Fibrosis in Endometriosis. *PLoS One* **8**, e76808 (2013).
640. Pabona, J. M. P. *et al.* Krüppel-like factor 9 and progesterone receptor coregulation of decidualizing endometrial stromal cells: Implications for the pathogenesis of endometriosis. *J. Clin. Endocrinol. Metab.* **97**, (2012).
641. Aghajanova, L. *et al.* The protein kinase A pathway-regulated transcriptome of endometrial stromal fibroblasts reveals compromised differentiation and persistent proliferative potential in endometriosis. *Endocrinology* **151**, 1341–1355 (2010).
642. Cheng, C. wen, Smith, S. K. & Charnock-Jones, D. S. Transcript profile and localization of Wnt signaling-related molecules in human endometrium. *Fertil. Steril.* **90**, 201–204 (2008).
643. Matsuzaki, S. DNA microarray analysis in endometriosis for development of more effective targeted therapies. *Front. Biosci. - Elit.* **3 E**, 1139–1153 (2011).
644. Zhang, L. *et al.* Intracellular Wnt/beta-catenin signaling underlying 17beta-estradiol-induced matrix metalloproteinase 9 expression in human endometriosis. *Biol. Reprod.* **94**, 70–71 (2016).
645. Zhang, L., Xiong, W., Xiong, Y., Liu, H. & Liu, Y. 17 β-Estradiol promotes vascular endothelial growth factor expression via the Wnt/β-catenin pathway during the pathogenesis of endometriosis. *Mol. Hum. Reprod.* **22**, 526–535 (2016).
646. Li, J., Dai, Y., Zhu, H., Jiang, Y. & Zhang, S. Endometriotic mesenchymal stem cells significantly promote fibrogenesis in ovarian endometrioma through the Wnt/β-catenin pathway by paracrine production of TGF-β1 and Wnt1. *Hum. Reprod.* **31**, 1224–1235 (2016).
647. Sumathi, V. P. & McCluggage, W. G. CD10 is useful in demonstrating endometrial stroma at ectopic sites and in confirming a diagnosis of endometriosis. *J. Clin. Pathol.* **55**, 391–392 (2002).
648. Huhtinen, K. *et al.* Intra-tissue steroid profiling indicates differential progesterone and testosterone metabolism in the endometrium and endometriosis lesions. *J. Clin. Endocrinol. Metab.* **99**, E2188–E2197 (2014).

649. Huhtinen, K. *et al.* Endometrial and endometriotic concentrations of estrone and estradiol are determined by local metabolism rather than circulating levels. *J. Clin. Endocrinol. Metab.* **97**, 4228–4235 (2012).
650. Wang, Y., Nicholes, K. & Shih, I. M. The Origin and Pathogenesis of Endometriosis. *Annu. Rev. Pathol. Mech. Dis.* **15**, 71–95 (2020).
651. Kvaskoff, M., Mesrine, S., Clavel-Chapelon, F. & Boutron-Ruault, M.-C. Endometriosis risk in relation to naevi, freckles and skin sensitivity to sun exposure: the French E3N cohort. *Int. J. Epidemiol.* **38**, 1143–1153 (2009).
652. Culley, L. *et al.* The social and psychological impact of endometriosis on women's lives: A critical narrative review. *Hum. Reprod. Update* **19**, 625–639 (2013).
653. Moradi, M., Parker, M., Sneddon, A., Lopez, V. & Ellwood, D. Impact of endometriosis on women's lives: A qualitative study. *BMC Womens. Health* **14**, 123 (2014).
654. Soliman, A. M. *et al.* The effect of endometriosis symptoms on absenteeism and presenteeism in the workplace and at home. *J. Manag. Care Spec. Pharm.* **23**, 745–754 (2017).
655. Soliman, A. M., Surrey, E., Bonafede, M., Nelson, J. K. & Castelli-Haley, J. Real-World Evaluation of Direct and Indirect Economic Burden Among Endometriosis Patients in the United States. *Adv. Ther.* **35**, 408–423 (2018).
656. National Institute for Health and Care Excellence (NICE). Endometriosis: diagnosis and management Guidance NICE guideline [NG73]. https://www.nice.org.uk/guidance/ng73 (2017).
657. Hager, M. *et al.* The Prevalence of Incidental Endometriosis in Women Undergoing Laparoscopic Ovarian Drilling for Clomiphene-Resistant Polycystic Ovary Syndrome: A Retrospective Cohort Study and Meta-Analysis. *J. Clin. Med.* **8**, 1210 (2019).
658. Rawson, J. M. R. Prevalence of endometriosis in asymptomatic women. *J. Reprod. Med. Obstet. Gynecol.* **36**, 513–515 (1991).
659. Thomas, E. J. The relevance of asymptomatic endometriosis. in *Human Reproduction* vol. 11 103–109 (Oxford University Press, 1996).
660. Soliman, A. M., Fuldeore, M. & Snabes, M. C. Factors Associated with Time to Endometriosis Diagnosis in the United States. *J. Women's Heal.* **26**, 788–797 (2017).
661. Hudelist, G. *et al.* Diagnostic delay for endometriosis in Austria and Germany: Causes and possible consequences. *Hum. Reprod.* **27**, 3412–3416 (2012).
662. Husby, G. K., Haugen, R. S. & Moen, M. H. Diagnostic delay in women with pain and endometriosis. *Acta Obstet. Gynecol. Scand.* **82**, 649–653 (2003).
663. Staal, A. H. J., Van Der Zanden, M. & Nap, A. W. Diagnostic Delay of Endometriosis in the Netherlands. *Gynecol. Obstet. Invest.* **81**, 321–324 (2016).
664. Ballard, K., Lowton, K. & Wright, J. What's the delay? A qualitative study of women's experiences of reaching a diagnosis of endometriosis. *Fertil. Steril.* **86**, 1296–1301 (2006).
665. DiVasta, A. D., Vitonis, A. F., Laufer, M. R. & Missmer, S. A. Spectrum of symptoms in women diagnosed with endometriosis during adolescence vs adulthood. *Am. J. Obstet. Gynecol.* **218**, 324.e1-324.e11 (2018).
666. Marasinghe, J. P. *et al.* History, pelvic examination findings and mobility of ovaries as a sonographic marker to detect pelvic adhesions with fixed ovaries. *J. Obstet. Gynaecol. Res.* **40**, 785–790 (2014).
667. *A Question of Balance*. (National Academies Press, 1999). doi:10.17226/9692.
668. Raleigh, V. S., Cooper, J., Bremner, S. A. & Scobie, S. Patient safety indicators for England from hospital administrative data: Case-control analysis and comparison with US data. *Bmj* **337**, 1219–1222 (2008).
669. Kodeda, K. *et al.* Population-based data from the Swedish Colon Cancer Registry. *Br. J. Surg.* **100**, 1100–1107 (2013).
670. Kelly, M. & Lamah, M. Evaluating the accuracy of data entry in a regional colorectal cancer database: Implications for national audit. *Color. Dis.* **9**, 337–339 (2007).

671. van Melle, W. MYCIN: a knowledge-based consultation program for infectious disease diagnosis. *Int. J. Man. Mach. Stud.* **10**, 313–322 (1978).
672. Davenport, T. & Kalakota, R. The potential for artificial intelligence in healthcare. *Futur. Healthc. J.* **6**, 94–98 (2019).
673. The AI Advantage: How to Put the Artificial Intelligence Revolution to Work - Thomas H. Davenport - Google Books. https://books.google.fi/books?hl=en&lr=&id=QzNwDwAAQBAJ&oi=fnd&pg=PR5&ots=FC9jUuURtS&sig=RvsyMPg0oCAl_WN839TztbgbjOc&redir_esc=y#v=onepage&q&f=false.
674. Khanna, S., Sattar, A. & Hansen, D. Artificial intelligence in health - The three big challenges. *Australasian Medical Journal* vol. 6 315–317 (2013).
675. Dreyer, K. & Allen, B. Artificial Intelligence in Health Care: Brave New World or Golden Opportunity? *J. Am. Coll. Radiol.* **15**, 655–657 (2018).
676. Haenssle, H. A. *et al.* Man against Machine: Diagnostic performance of a deep learning convolutional neural network for dermoscopic melanoma recognition in comparison to 58 dermatologists. *Ann. Oncol.* **29**, 1836–1842 (2018).
677. Laï, M. C., Brian, M. & Mamzer, M. F. Perceptions of artificial intelligence in healthcare: Findings from a qualitative survey study among actors in France. *J. Transl. Med.* **18**, 14 (2020).
678. Ahmed, Z., Mohamed, K., Zeeshan, S. & Dong, X. Q. Artificial intelligence with multi-functional machine learning platform development for better healthcare and precision medicine. *Database* vol. 2020 (2020).
679. Sboner, A. & Elemento, O. A primer on precision medicine informatics. *Brief. Bioinform.* **17**, 145–153 (2016).
680. Ritchie, M. D., de Andrade, M. & Kuivaniemi, H. The foundation of precision medicine: Integration of electronic health records with genomics through basic, clinical, and translational research. *Front. Genet.* **6**, 104 (2015).
681. Karczewski, K. J. & Snyder, M. P. Integrative omics for health and disease. *Nat. Rev. Genet.* **19**, 299–310 (2018).
682. Raghupathi, W. & Raghupathi, V. Big data analytics in healthcare: promise and potential. *Heal. Inf. Sci. Syst.* **2**, 1–10 (2014).
683. Beam, A. L. & Kohane, I. S. Big data and machine learning in health care. *JAMA - Journal of the American Medical Association* vol. 319 1317–1318 (2018).
684. Alyass, A., Turcotte, M. & Meyre, D. From big data analysis to personalized medicine for all: Challenges and opportunities. *BMC Medical Genomics* vol. 8 33 (2015).
685. Koninckx, P. R., Meuleman, C., Oosterlynck, D. & Cornillie, F. J. Diagnosis of deep endometriosis by clinical examination during menstruation and plasma CA-125 concentration. *Fertil. Steril.* **65**, 280–287 (1996).
686. Berkley, K. J., Rapkin, A. J. & Papka, R. E. The pains of endometriosis. *Science* vol. 308 1587–1589 (2005).
687. Giudice, L. C. & Kao, L. C. Endometriosis. in *Lancet* vol. 364 1789–1799 (2004).
688. Bordin, L. *et al.* Evaluation of erythrocyte band 3 phosphotyrosine level, glutathione content, CA-125, and human epididymal secretory protein E4 as combined parameters in endometriosis. *Fertil. Steril.* **94**, 1616–1621 (2010).
689. Cheng, Y. M., Wang, S. T. & Chou, C. Y. Serum CA-125 in preoperative patients at high risk for endometriosis. *Obstet. Gynecol.* **99**, 375–380 (2002).
690. Medl, M. *et al.* Serum levels of the tumour-associated trypsin inhibitor in patients with endometriosis. *BJOG An Int. J. Obstet. Gynaecol.* **104**, 78–81 (1997).
691. Fedele, L. *et al.* Serum Ca-125 concentrations in endometriosis. *Acta Eur. Fertil.* **20**, 137–139 (1989).
692. Pittaway, D. E. & Fayez, J. A. The use of CA-125 in the diagnosis and management of endometriosis. *Fertil. Steril.* **46**, 790–795 (1986).

693. Barbieri, R. L. *et al.* Elevated serum concentrations of CA-125 in patients with advanced endometriosis. *Fertil. Steril.* **45**, 630–634 (1986).
694. Bast, R. C. *et al.* A Radioimmunoassay Using a Monoclonal Antibody to Monitor the Course of Epithelial Ovarian Cancer. *N. Engl. J. Med.* **309**, 883–887 (1983).
695. Kitawaki, J. *et al.* Usefulness and limits of CA-125 in diagnosis of endometriosis without associated ovarian endometriomas. *Hum. Reprod.* **20**, 1999–2003 (2005).
696. Moore, R. G. *et al.* Serum HE4 levels are less frequently elevated than CA125 in women with benign gynecologic disorders. *Am. J. Obstet. Gynecol.* **206**, 351.e1-351.e8 (2012).
697. Moore, R. G. *et al.* The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. *Gynecol. Oncol.* **108**, 402–408 (2008).
698. Huhtinen, K. *et al.* Serum HE4 concentration differentiates malignant ovarian tumours from ovarian endometriotic cysts. *British Journal of Cancer* (2009). doi:10.1038/sj.bjc.6605011.
699. Koninckx, P. R., Riittinen, L., Seppala, M. & Cornillie, F. J. CA-125 and placental protein 14 concentrations in plasma and peritoneal fluid of women with deeply infiltrating pelvic endometriosis. in *Fertility and Sterility* vol. 57 523–530 (Elsevier, 1992).
700. Matalliotakis, I. M., Goumenou, A. G., Mulayim, N., Karkavitsas, N. & Koumantakis, E. E. High concentrations of the CA-125, CA 19-9 and CA 15-3 in the peritoneal fluid between patients with and without endometriosis. *Arch. Gynecol. Obstet.* **271**, 40–45 (2005).
701. do Amaral, V. F. *et al.* Positive correlation between serum and peritoneal fluid CA-125 levels in women with pelvic endometriosis. *Sao Paulo Med. J.* **124**, 223–227 (2006).
702. Kvaskoff, M. *et al.* Endometriosis: A high-risk population for major chronic diseases? *Hum. Reprod. Update* **21**, 500–516 (2014).
703. Gupta, S. *et al.* Serum and peritoneal abnormalities in endometriosis: Potential use as diagnostic markers. *Minerva Ginecol.* **58**, 527–551 (2006).
704. Patton, P. E., Field, C. S., Harms, R. W. & Coulam, C. B. CA-125 levels in endometriosis. *Fertil. Steril.* **45**, 770–773 (1986).
705. Hornstein, M. D., Thomas, P. P., Gleason, R. E. & Barbieri, R. L. Menstrual cyclicity of CA-125 in patients with endometriosis. *Fertil. Steril.* **58**, 279–283 (1992).
706. O'Shaughnessy, A., Check, J. H., Nowroozi, K. & Lurie, D. CA 125 levels measured in different phases of the menstrual cycle in screening for endometriosis. *Obstet. Gynecol.* **81**, 99–103 (1993).
707. Wang, Y., van der Zee, M., Fodde, R. & Blok, L. J. Wnt/B-catenin and sex hormone signaling in endometrial homeostasis and cancer. *Oncotarget* vol. 1 674–684 (2010).
708. Wang, Y. *et al.* Progesterone inhibition of Wnt/β-catenin signaling in normal endometrium and endometrial cancer. *Clin. Cancer Res.* **15**, 5784–5793 (2009).
709. Tulac, S. *et al.* Identification, characterization, and regulation of the canonical Wnt signaling pathway in human endometrium. *J. Clin. Endocrinol. Metab.* **88**, 3860–3866 (2003).
710. Kiewisz, J., Wasniewski, T. & Kmiec, Z. Participation of WNT and β -catenin in physiological and pathological endometrial changes: Association with angiogenesis. *BioMed Research International* vol. 2015 (2015).
711. Wagner, J. & Lehmann, L. Estrogens modulate the gene expression of Wnt-7a in cultured endometrial adenocarcinoma cells. in *Molecular Nutrition and Food Research* vol. 50 368–372 (Mol Nutr Food Res, 2006).
712. Oehler, M. K., MacKenzie, I. Z., Rees, M. C. P., Bicknell, R. & Wallwiener, D. Wnt-7a is upregulated by norethisterone in human endometrial epithelial cells: A possible mechanism by which progestogens reduce the risk of estrogen-induced endometrial neoplasia. *Cancer Lett.* **186**, 75–81 (2002).
713. Banerjee, S. *et al.* WISP-2 gene in human breast cancer: Estrogen and progesterone inducible expression and regulation of tumor cell proliferation. *Neoplasia* **5**, 63–73 (2003).
714. Saegusa, M., Hashimura, M., Yoshida, T. & Okayasu, I. B-Catenin Mutations and Aberrant Nuclear Expression During Endometrial Tumorigenesis. *Br. J. Cancer* **84**, 209–217 (2001).

715. Brabletz, T. *et al.* Variable β-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 10356–10361 (2001).
716. Kudo, Y. *et al.* Invasion and metastasis of oral cancer cells require methylation of E-cadherin and/or degradation of membranous β-catenin. *Clin. Cancer Res.* **10**, 5455–5463 (2004).
717. Techasen, A. *et al.* Loss of E-cadherin promotes migration and invasion of cholangiocarcinoma cells and serves as a potential marker of metastasis. *Tumor Biol.* **35**, 8645–8652 (2014).
718. Esteve, P. *et al.* Secreted frizzled-related proteins are required for Wnt/β-catenin signalling activation in the vertebrate optic cup. *Development* **138**, 4179–4184 (2011).
719. Fontenot, E. *et al.* A novel monoclonal antibody to secreted frizzled-related protein 2 inhibits tumor growth. *Mol. Cancer Ther.* **12**, 685–695 (2013).
720. Gehmert, S., Sadat, S., Song, Y. H., Yan, Y. & Alt, E. The anti-apoptotic effect of IGF-1 on tissue resident stem cells is mediated via PI3-kinase dependent secreted frizzled related protein 2 (Sfrp2) release. *Biochem. Biophys. Res. Commun.* **371**, 752–755 (2008).
721. Kim, M., Han, J. H., Kim, J. H., Park, T. J. & Kang, H. Y. Secreted frizzled-related protein 2 (sFRP2) functions as a melanogenic stimulator; The role of sFRP2 in UV-induced hyperpigmentary disorders. *J. Invest. Dermatol.* **136**, 236–244 (2016).
722. Kwack, M. H. *et al.* SFRP2 augments Wnt/β-catenin signalling in cultured dermal papilla cells. *Experimental Dermatology* vol. 25 813–815 (2016).
723. Roth, W. *et al.* Secreted Frizzled-related proteins inhibit motility and promote growth of human malignant glioma cells. *Oncogene* **19**, 4210–4220 (2000).
724. Sugiyama, Y. *et al.* Sfrp1 and Sfrp2 are not involved in Wnt/β-catenin signal silencing during lens induction but are required for maintenance of Wnt/β-catenin signaling in lens epithelial cells. *Dev. Biol.* **384**, 181–193 (2013).
725. Xiao, X. *et al.* Promoting roles of the secreted frizzled-related protein 2 as a Wnt agonist in lung cancer cells. *Oncol. Rep.* **34**, 2259–2266 (2015).
726. Yamamura, S. *et al.* Oncogenic functions of secreted Frizzled-related protein 2 in human renal cancer. *Mol. Cancer Ther.* **9**, 1680–1687 (2010).
727. Vercellini, P., Viganò, P., Somigliana, E. & Fedele, L. Endometriosis: Pathogenesis and treatment. *Nat. Rev. Endocrinol.* **10**, 261–275 (2014).
728. Burney, R. O. & Giudice, L. C. Pathogenesis and pathophysiology of endometriosis. *Fertility and Sterility* vol. 98 511–519 (2012).
729. Berlanda, N., Vercellini, P. & Fedele, L. The outcomes of repeat surgery for recurrent symptomatic endometriosis. *Current Opinion in Obstetrics and Gynecology* vol. 22 320–325 (2010).
730. Hayata, T., Matsu, T., Kawano, Y., Matsui, N. & Miyakawa, I. Scanning electron microscopy of endometriotic lesions in the pelvic peritoneum and the histogenesis of endometriosis. *Int. J. Gynecol. Obstet.* **39**, 311–319 (1992).
731. Howard, F. M. The role of laparoscopy as a diagnostic tool in chronic pelvic pain. *Bailliere's Best Pract. Res. Clin. Obstet. Gynaecol.* **14**, 467–494 (2000).
732. Howard, F. M. The role of laparoscopy in chronic pelvic pain: Promise and pitfalls. *Obstetrical and Gynecological Survey* vol. 48 357–387 (1993).
733. Vignali, M. *et al.* Surgical treatment of deep endometriosis and risk of recurrence. *J. Minim. Invasive Gynecol.* **12**, 508–513 (2005).
734. Rizk, B. *et al.* Recurrence of endometriosis after hysterectomy. *Facts, views Vis. ObGyn* **6**, 219–27 (2014).
735. Fedele, L. *et al.* Long-term follow-up after conservative surgery for bladder endometriosis. *Fertil. Steril.* **83**, 1729–1733 (2005).
736. Cao, Q., Lu, F., Feng, W. W., Ding, J. X. & Hua, K. Q. Comparison of complete and incomplete excision of deep infiltrating endometriosis. *Int. J. Clin. Exp. Med.* **8**, 21497–21506 (2015).
737. Vasquez, G., Cornillie, F. & Brosens, I. A. Peritoneal endometriosis: Scanning electron microscopy and histology of minimal pelvic endometriotic lesions. *Fertil. Steril.* **42**, 696–703 (1984).

738. Donnez, J. & Van Langendonckt, A. Typical and subtle atypical presentations of endometriosis. *Current Opinion in Obstetrics and Gynecology* vol. 16 431–437 (2004).
739. Murphy, A. A., Green, W. R., Bobbie, D., dela Cruz, Z. C. & Rock, J. A. Unsuspected endometriosis documented by scanning electron microscopy in visually normal peritoneum. *Fertil. Steril.* **46**, 522–524 (1986).
740. Cheong, Y. C. *et al.* Peritoneal healing and adhesion formation/reformation. *Hum. Reprod. Update* **7**, 556–566 (2001).
741. Rauh-Hain, J. A. & Laufer, M. R. Increased diagnostic accuracy of laparoscopy in endometriosis using indigo carmine: A new technique. *Fertil. Steril.* **95**, 1113–1114 (2011).
742. Lessey, B. A., Higdon, H. L., Miller, S. E. & Price, T. A. Intraoperative detection of subtle endometriosis: a novel paradigm for detection and treatment of pelvic pain associated with the loss of peritoneal integrity. *J. Vis. Exp.* 4313 (2012) doi:10.3791/4313.
743. Manhes, H., Shulman, A., Haag, T., Canis, M. & Demontmarin, J. L. Infertility due to diseased pelvic peritoneum: Laparoscopic treatment. *Gynecol. Obstet. Invest.* **37**, 191–195 (1994).
744. Potlog-Nahari, C. *et al.* CD10 immunohistochemical staining enhances the histological detection of endometriosis. *Fertil. Steril.* **82**, 86–92 (2004).
745. Groisman, G. M. & Meir, A. CD10 is helpful in detecting occult or inconspicuous endometrial stromal cells in cases of presumptive endometriosis. *Arch. Pathol. Lab. Med.* **127**, 1003–1006 (2003).
746. As-Sanie, S. *et al.* Assessing research gaps and unmet needs in endometriosis. *American Journal of Obstetrics and Gynecology* vol. 221 86–94 (2019).
747. Kinkel, K., Frei, K. A., Balleyguier, C. & Chapron, C. Diagnosis of endometriosis with imaging: A review. *European Radiology* vol. 16 285–298 (2006).
748. Y Weintraub, A. Think Endometriosis: Delay in Diagnosis or Delay in Referral to Adequate Treatment? *J. Fertil. Vitr. - IVF-Worldwide, Reprod. Med. Genet. Stem Cell Biol.* **02**, (2014).
749. Brosens, I. & Benagiano, G. Poor results after surgery for rectovaginal endometriosis can be related to uterine adenomyosis. *Human Reproduction* vol. 27 3360–3361 (2012).
750. Balleyguier, C., Chapron, C., Chopin, N., Hélénon, O. & Menu, Y. Abdominal wall and surgical scar endometriosis: Results of magnetic resonance imaging. *Gynecol. Obstet. Invest.* **55**, 220–224 (2003).
751. Takeuchi, H. *et al.* A novel technique using magnetic resonance imaging jelly for evaluation of rectovaginal endometriosis. *Fertil. Steril.* **83**, 442–447 (2005).
752. Chamié, L. P., Blasbalg, R., Ricar-do Mendes, A. P., Warmbrand, G. & Serafini, P. C. Findings of pelvic endo-metriosis at transvaginal US, MR imaging, and laparoscopy. *Radiographics* **31**, (2011).
753. Agrawal, S. *et al.* The miRNA mirage: How close are we to finding a non-invasive diagnostic biomarker in endometriosis? a systematic review. *Int. J. Mol. Sci.* **19**, 599 (2018).
754. Vodolazkaia, A. *et al.* Evaluation of a panel of 28 biomarkers for the non-invasive diagnosis of endometriosis. *Hum. Reprod.* **27**, 2698–2711 (2012).
755. Schreinemacher, M. H. *et al.* Towards endometriosis diagnosis by gadofosveset-trisodium enhanced magnetic resonance imaging. *PLoS One* **7**, (2012).
756. Wood, C., Kuhn, R. & Tsaltas, J. Laparoscopic diagnosis of endometriosis. *Aust. New Zeal. J. Obstet. Gynaecol.* **42**, 277–281 (2002).
757. Mettler, L. *et al.* Accuracy of laparoscopic diagnosis of endometriosis. *JSLS* **7**, 15–18 (2003).
758. Walter, A. J., Hentz, J. G., Magtibay, P. M., Cornella, J. L. & Magrina, J. F. Endometriosis: Correlation between histologic and visual findings at laparoscopy. in *American Journal of Obstetrics and Gynecology* vol. 184 1407–1413 (Mosby Inc., 2001).
759. Hori, Y. Diagnostic laparoscopy guidelines : This guideline was prepared by the SAGES Guidelines Committee and reviewed and approved by the Board of Governors of the Society of American Gastrointestinal and Endoscopic Surgeons (SAGES), November 2007. *Surgical endoscopy* vol. 22 1353–1383 (2008).

760. Chapron, C. *et al.* Questioning patients about their adolescent history can identify markers associated with deep infiltrating endometriosis. *Fertil. Steril.* **95**, 877–881 (2011).
761. Steenberg, C. K., Tanbo, T. G. & Qvigstad, E. Endometriosis in adolescence: Predictive markers and management. *Acta Obstet. Gynecol. Scand.* **92**, 491–495 (2013).
762. Geysenbergh, B., Dancet, E. A. F. & D'hooghe, T. Detecting endometriosis in adolescents: why not start from self-report screening questionnaires for adult women? *Gynecol. Obstet. Invest.* **82**, 322–328 (2017).
763. Bush, D., Brick, E., East, M. C. & Johnson, N. Endometriosis education in schools: A New Zealand model examining the impact of an education program in schools on early recognition of symptoms suggesting endometriosis. *Aust. New Zeal. J. Obstet. Gynaecol.* **57**, 452–457 (2017).
764. Rey, J. P. & Ellies, D. L. Wnt modulators in the biotech pipeline. *Developmental Dynamics* vol. 239 102–114 (2010).
765. Lu, B., Green, B. A., Farr, J. M., Lopes, F. C. M. & Van Raay, T. J. Wnt drug discovery: Weaving through the screens, patents and clinical trials. *Cancers* vol. 8 (2016).

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

Painosalama, Turku, Finland 2021

ISBN 978-951-29-8712-2 (PRINT)
ISBN 978-951-29-8713-9 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)