Testing dual mating strategy hypothesis using two potential secondary sexual characteristics, androstadienone and facial masculinity

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Master`s thesis

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Psychology

June 2020

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UNIVERSITY OF TURKU

Department of Psychology and Speech Language Pathology / Faculty of Social Sciences

SOINI, EETU:

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Master´s thesis: 36 p.
Psychology
June 2020

Dual mating strategy hypothesis has been influential in research of human mate choice for twenty years. According to dual mating hypothesis women prefer different attributes in men in different phases of menstrual cycle. This may have been adaptive as it can lead to situation where the offspring is fathered by genetically strong male, who may not be willing to invest in the offspring and women may have gotten help in rearing the offspring from another man, not knowing that he is not the biological father. Although first studies found support for the hypothesis, recently it has been challenged as earlier studies reporting significant findings have been criticized for their methodology and studies, using larger sample sizes in comparison with earlier studies, have failed to find support for dual mating hypothesis. In the current study dual mating strategy hypothesis was tested using two potential cues of genetic quality, facial masculinity and putative human chemosignal androstadienone. Current study was conducted as placebo controlled, double-blind between-subjects design. Eighty-one (81) self-reported heterosexual females differing in fertility status (fertile phase of menstrual cycle n=17, non-fertile phase of menstrual cycle n=30 and use of hormonal contraceptives n=34) participated in the study. Participants task was twofold: first to rate attractiveness of individual pictures of male faces, and to choose the more attractive face from morphed face pairs. Both evaluations were done twice, once before and once after the exposure to androstadienone or placebo treatment. Facial attractiveness was rated from individual pictures and masculinity preference was tested using forced choice task with face pairs consisting of two morphed versions of same face, one morphed to be more masculine and the other morphed to be more feminine. At half point of the rating task half of the participants received androstadienone (n=41) and half were in placebo condition (n=40) to test if androstadienone would increase perceived attractiveness. Analyses showed no preference shift towards masculinity in fertile compared to non-fertile phase of menstrual cycle, and no decrease in masculinity preference was found in hormonal contraceptive users as dual mating theory predicts. Also, no evidence was found that androstadienone increases female perceptions of male face attractiveness as some studies have found. Our results found no evidence for the link between women's preference for cues of masculinity and their hormonal status, adding up to the growing body of research failing to find support for dual mating strategy hypothesis. Regarding androstadienone we did not find support for its role as chemosignal of genetic quality, adding up to controversial field of results.

Keywords: Dual mating Hypothesis, Mate Choice, Androstadienone, Chemosignal, Masculinity Preference, Hormonal Contraception, Evolutionary psychology

1. INTRODUCTION

Human mate choice is a complex problem. Pool from which to choose a potential mate is nowadays seemingly infinite, desirable attributes are numerous and all the desired attributes are seldomly found in one individual, hence compromises are often made. Both sexes prefer healthy appearance, signs of intelligence and kindness in potential mate but there are also sex differences as males are more attracted to physical attributes, whereas women tend to prefer attributes linked to high social status (Buss, 1989). According to evolutionary psychology sexes differ in their potential reproductive capability and have thus faced different adaptive problems in evolutionary history. Different adaptive problems have led to different preferences in mate choice to solve these problems. From evolutionary point of view optimal strategy for males is more straightforward as there are fewer biological constraints on potential number of offspring, female mate choice is harder to explain as reproduction bears significant cost and hinders future reproduction. One of the theories tackling female mate choice from evolutionary perspective is dual mating theory, that suggests that to get the good genes that increase the survival or reproductive success of the offspring and to get support for rearing the offspring, women should prefer cues of genetic quality in the fertile phase of the menstrual cycle and prefer cues of parental investment in the non-fertile phase. On a special note, when referring to men and women in this thesis, biological sex determined by gamete size, is meant. Thus, "men" refers to sex with smaller gametes and "women" to sex with bigger gametes. No stand is taken regarding gender or gender identity as they are out of the scope of current study.

Choosing a mate has crucial role in reproductive success, as rearing an offspring itself is costly in terms of resources and time. Even though offspring inherits roughly half of his/her alleles from each parent, there lies a difference in parental invest, most notably women's cost of producing larger gametes, and pregnancy (Trivers, 1972). As this cost is greater for women, sexual strategies theory (Buss & Schmitt, 1993) predicts that women should prefer cues of both, genetic quality and parental investment in order to produce genetically as high quality offspring as possible and receive support in rearing

them. However, for men with high genetic quality optimal mating strategy is not to form a long-term relationship, but rather to have as many short-term relationships as possible, as benefit of potential offspring outweigh cost of reproduction (Buss & Schmitt, 1993). Sexes thus differ in their optimal mating strategy according to evolutionary psychology.

Dual mating strategy hypothesis suggests that in scenario described in previous chapter natural selection could have favoured women who during fertile phase of menstrual cycle prefer cues of good genes and in non-fertile phase cues of willingness to invest in offspring (Pillsworth & Haselton, 2006). Although in many primate species ovulation is advertised by for example sexual swelling, in humans no swelling, or other kind of advertisement, happens (Havlíček, Dvořáková, Bartoš, & Flegr, 2006). As human ovulation is "concealed" and conception is possible for only in a limited time window near ovulation, according to dual mating hypothesis this enables an alternative mating strategy to the conventional pair bonding. Women could get parental investment by forming a long-term relationship with individual willing to invest and obtaining the "good genes" via adultery during fertile phase of menstrual cycle with a male of high genetic value. It is noteworthy that to utilize this hypothetical strategy, women do not need make conscious decision, but rather subtle changes in behaviour near ovulation can be sufficient for obtaining the "good genes". Also, although dual mating theory predicts that female preference shifts depending on cycle phase, these preference shifts do not dictate human behaviour, but can be seen statistically at the level of population.

Evolutionary psychology differs from traditional psychology as it aims to explain psychological phenomena with ultimate explanations. Ultimate explanations aims to answer question why a trait exist, by explaining its adaptive importance in evolutionary history (Scott-Phillips, Dickins, & West, 2011). A trait that has increased either survival or reproductive rate has increased the fitness of the individual and hence been beneficial for passing on the genes. Traditional psychology aims to explain phenomena in proximate level, answering question like how does this phenomenon work. As ultimate and proximate explanations answer to different questions, they complement each other rather than compete with each other (Scott-Phillips et al.,

2011). For example proximate explanation why women prefer cues of genetic quality during fertile phase could be that men of high genetic quality are seen as attractive, whereas ultimate explanation for the same question could be that preference shift has been advantageous in evolutionary history and increased the quality of offspring, thus increasing the fitness of the woman. Dual mating theory attempts to explain why the preference shift has evolved and takes no stand on what mechanism causes the shift to happen. There are also various evolutionary (e.g. cost of getting caught) and social reasons not to cheat in relationship and most do not engage in adultery, cyclically or otherwise.

Testosterone dependent traits have been hypothesized to be honest signals of genetic quality in males, as they may signal immunocompetence (Folstad & Karter, 1992; Thornhill & Gangestad, 1996) and several studies have found support for dual mating strategy theory using testosterone dependent secondary sexual traits (Johnston, Hagel, Franklin, Fink, & Grammer, 2001; Penton-Voak & Perrett, 2000; Penton-Voak et al., 1999). Although dual mating hypothesis has been influential in research of human sexual selection, recently studies with more rigorous statistical methods and larger sample sizes in comparison with earlier studies have failed to replicate results supporting dual mating strategy hypothesis (Jones et al., 2018; Marcinkowska et al., 2016; Marcinkowska, Galbarczyk, & Jasienska, 2018; Stern, Gerlach, & Penke, 2020) and earlier results have been criticized for their methods (Gangestad et al., 2016; Jones, Hahn, & DeBruine, 2019). As there are mixed results in the field more studies are needed to address the controversy and to better understand psychology of human mate preference. In the current study dual mating hypothesis is tested using larger sample size in comparison with earlier studies reporting significant findings and using two different cues of genetic quality, androstadienone and facial masculinity.

1.1. Mate choice

Due to mate choices crucial part role in reproductive success it has been studied extensively in evolutionary psychology. Among species in which there exists asymmetry in cost of having an offspring, usually sex that invest more on the offspring tends to be “choosier” and the sex that invests less competes among members of the

same sex over mating opportunities with individuals of the other sex (Trivers, 1972). In most species, including humans, sex that produces larger gametes (females) tend to be choosier and sex with smaller sex cells (males) tend to compete with each other over access to the other sex, although this can be reversed if the sex with smaller sex cells invest more in the offspring (Trivers, 1972). Trivers (1972, p.55) has defined parental investment as "*any investment by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring*", as the investment can be done before or after parturition and can be anything from metabolic investment in form of gamete size to any behavior that aids offspring's survival, such as feeding or guarding.

As sexes differ in the mandatory investment in their offspring, their strategies for maximizing reproductive success differ from another (Buss & Schmitt, 1993). Hypothetically only limiting factor for men's reproductive success is the number of available fertile women, as the benefit of a potential offspring outweighs the meager cost of copulation. According to sexual strategies hypothesis using a short-term mating strategy can be beneficial for men in terms of fitness when compared to forming a long-term relationship and investing to an offspring. A father of one can double the amount of his offspring by using short term mating strategy without bearing the costs of rearing an offspring, assuming that the offspring from short-term mating survives without parental investment from father. Using short term mating strategy does not exclude the use of long-term mating strategy either alternatively, or at the same time.

For women the situation is more complicated as copulation can lead to pregnancy, which is costly, has risks and hinders future reproduction for as many as several years (Buss & Schmitt, 1993). Thus, as the minimal parental investment for women is significantly larger, women should invest in the offspring quality and the resources that a man can provide to alleviate the costs of childbearing (Buss & Schmitt, 1993). Sexual strategy theory predicts that women should prefer both signals of willingness to invest in offspring and to cues of genetic quality.

It is worth mentioning that the above are generalization, and men can, and indeed do invest heavily in offspring compared to other mammals (Buss & Schmitt, 1993; Geary, 2000). After birth men can invest in the offspring by protecting and provisioning, and

variation in investment within both sexes is considerable (Buss & Schmitt, 1993). Hence, even though men and women differ in parental investment, differences are smaller compared to other mammals.

1.2. Potential cues of male reproductive fitness

Masculine traits are potential cues for male reproductive fitness as testosterone may have immunosuppressive effect (Folstad & Karter, 1992; Thornhill & Gangestad, 1996; but for controversial results see Nowak, Pawłowski, Borkowska, Augustyniak, & Drulis-Kawa, 2018). Rationale here is that testosterone dependent secondary sexual characteristics are honest signals of health and genetic quality. Honest signals are traits that convey useful information from the signaler to the recipient but also bear a cost for the signaler (Lindström & Kotiaho, 2002). If signaling is costly for the signaler, those with better genetic quality are able to exhibit these traits to a greater extend compared to individual with lower genetic quality as individuals with lower genetic quality are unable to bear the cost inflicted from the trait. As the trait cannot be used for deceivable signaling, it can be used for example assessing quality of a potential mate (Zahavi, 1975), like peahen evaluates suitor by the size of its tail feathers.

As testosterone may have immunosuppressive effect, those with sound immune system are able to exhibit more prominent secondary sexual characteristics, making these traits honest signals of immunocompetence (Thornhill & Gangestad, 1996) and some of these secondary sexual characteristics traits have been linked to health (Foo, Simmons, & Rhodes, 2017; Rhodes, Chan, Zebrowitz, & Simmons, 2003).

1.2.1. Facial attractiveness and dimorphism

Differences between average male and female faces can be attributed to different levels of sex hormones. These differences start to show in puberty (Rhodes, 2006). Males typically have a larger jaw, cheekbones and brow ridges which are linked to testosterone, whereas in females aforementioned characteristics are smaller and linked to estrogen (Rhodes, 2006). Facial attractiveness has been hypothesized to

signal fertility in females (Law Smith et al., 2006), and health in males (Foo et al., 2017; Rantala et al., 2012; Rhodes et al., 2003; Thornhill & Gangestad, 2006) although there is inconsistency in the reported strength of the link (Foo et al., 2017; Rantala et al., 2012).

First studies regarding women's preference for facial dimorphism reported that women preferred more feminine faces (Penton-Voak et al., 1999; Perrett et al., 1998), but later studies found the opposite result (Foo et al., 2017; Johnston et al., 2001; Jones et al., 2018). In addition to levels of sex hormones male faces may also signal other characteristics relevant to mate selection. Facial masculinity has been linked to decreased perceived and actual interest in infants (Roney, Hanson, Durante, & Maestripieri, 2006) and less commitment into a relationship (Boothroyd, Jones, Burt, & Perrett, 2007).

1.2.2. Chemosignals

Chemosignals are chemical compounds (molecule or mixture of molecules) (Wyatt, 2014) that transmit information from releaser to receiver. Benefits of chemical communication include low cost of production compared to other modalities and variation in signal duration. Social communication via chemical signals is important in most mammals (Petrulis, 2013) and is known to affect behaviors including reproduction, maternal care/ parent-offspring communication, aggression and alarm responses (Petrulis, 2013). Although social chemosignaling is widely accepted in other mammals, there is ongoing debate whether humans possess an ability to communicate with chemosignals and what kind of role does this kind of communication have in social interaction.

Some chemosignals (some formerly called pheromones) are thought to signal mate quality. In humans there exist sex differences in relation to chemosignals, as men have larger apocrine glands (Doty, Green, Ram, & Yankell, 1982), but women have 75% more apocrine glands (Brody, 1975). The amount of microbes in axillae begins to differ in puberty; men tend to have more microbes than women (Marples, 1982; Somerville, 1969). Men's sweat also contains more androstadienone and androstanol (Gower & Ruparelia, 1993), that are derivates of testosterone. The fact that these glands become

active at puberty, same time as axillary and pubic hair start to grow, supports the idea that these compounds may be used for chemical communication (Lübke & Pause, 2015; Wyatt, 2015).

As male chemosignals are thought to be derivatives of testosterone (for example androstadienone) or to include derivatives of testosterone, they have been studied in mate choice context as honest signals. Male chemosignals have been shown to increase women's evaluations of male attractiveness (Ferdenzi, Delplanque, Atanassova, & Sander, 2016; Saxton, Lyndon, Little, & Roberts, 2008; Thorne, Neave, Scholey, Moss, & Fink, 2002) but not all studies have been able to find the effect (Hare, Schlatter, Rhodes, & Simmons, 2017). It is also noteworthy that in study conducted by Ferdenzi et al. (2016) androstadienone also increased male evaluations of female face attractiveness, and thus effect may not be sex specific.

1.2.3. Dark side of masculinity

Masculinity has been linked to various traits that are beneficial for the mate choice, but there are also drawbacks as masculinity may negatively affect parental investment. Males that exhibit strong physical signs of masculinity report more infidelity (Rhodes, Morley, & Simmons, 2013), show preference for short-term relationships (Arnocky et al., 2018) and are perceived to have decreased qualities as a parent (Perrett et al., 1998). In addition, masculinity has been linked to increased competitiveness (Archer, 2009) and dominance (Geniole, Denson, Dixson, Carré, & McCormick, 2015), which can be advantageous in intrasexual competition, and thus be desirable attributes for an offspring, but are not necessarily desirable traits in long-term partner.

Thus, it seems that women face problem in mate choice, where potential "good genes" for the offspring are accompanied by undesirable behavior traits in the father. As optimal mating strategy for men differs from that of women, according to sexual strategy theory (Buss & Schmitt, 1993) male with high genetic quality should favor short term mating strategy, and thus be willing to invest in the offspring in terms of good genes, but be reluctant to invest in the offspring in the long term. Likewise, male with lesser genetic quality cannot utilize short-term mating strategy, and hence be

willing to invest in the offspring through long-term investments, such as provisioning and protection.

1.3. Dual mating strategy

In the situation described in earlier chapter natural selection could have favored a female who formed a long-term relationship with a male willing to invest but obtained good genes outside the relationship. Dual mating strategy (Pillsworth & Haselton, 2006) suggests that to gain both, good genes and parental investment, women should prefer cues of genetic quality (including masculinity) near ovulation when conception is likely and prefer more feminine traits in other phases of menstrual cycle. Thus, women could get the parental investment from the long-term relationship, and the good genes from another male via adultery, though strategy like this has its risks, as infidelity may end the long-term relationship leaving women without parental investment. Main point of the theory is that slight changes in behavior could (theoretically) maximize the fitness of women and offspring. It should be emphasized that ultimate level explanations answer questions regarding the reason why a trait has evolved and what adaptive problem it solves. Ultimate level explanations should not be used to explain individual behavior and no moral judgements should be made based on ultimate level explanation.

Near ovulation women are shown to prefer masculine faces (Johnston et al., 2001; Penton-Voak & Perrett, 2000; Penton-Voak et al., 1999), bodily masculinity (Little, Jones, & Burriss, 2007), voices of more masculine males (Feinberg et al., 2006) and odors of self-reported dominant males (Havlicek, Roberts, & Flegr, 2005). Additional evidence for the theory comes from studies that have linked the preference shift towards masculinity to hormonal changes associated to menstrual cycle, such as estradiol (Johnston et al., 2001; Roney & Simmons, 2008; Roney, Simmons, & Gray, 2011), testosterone (Lisa L. M. Welling, Jones, DeBruine, et al., 2008) and cortisol levels (Ditzen, Palm-Fischbacher, Gossweiler, Stucky, & Ehlert, 2017), which all fluctuate during menstrual cycle. Thus, it can be hypothesized that these changes in preferences for facial dimorphism are caused by hormonal fluctuations.

Dual mating strategy has also consequences regarding use of hormonal contraceptives, as hormonal contraceptives can “smoothen” hormonal variation (Alvergne & Lummaa, 2010). Some studies have found that women using hormonal contraceptives do not show preference for facial or vocal masculinity (Feinberg, DeBruine, Jones, & Little, 2008; Little, Jones, Penton-Voak, Burt, & Perrett, 2002; Penton-Voak et al., 1999), as normally ovulating women do, but others have reported contradictory results (Cobey, Little, & Roberts, 2015; Jones et al., 2019). It has even been proposed that use of hormonal contraceptives may cause problems in relationship. If hormonal contraceptive use affects masculinity preference, either starting or stopping the use of hormonal contraceptives during relationship would change the mate preference, potentially leading to dissatisfaction in current relationship (Roberts et al., 2014).

1.3.1. Critique of dual mating hypothesis

Although dual mating strategy theory has been influential regarding women’s mating tactics, recently there has been critique of the methodology of earlier studies regarding dual mating theory. Jones et. al. (2019) argue that earlier studies have used too small of a sample size in addition to using self-reported information regarding menstrual cycle and between subjects design. For example earlier studies linking cycle phase and increased preference for masculinity have had sample size smaller than 50 (Johnston et al., 2001; Penton-Voak et al., 1999) or have used only one set of pictures to evaluate masculinity preference (Penton-Voak & Perrett, 2000). According to Gangestad et al. (2016) the small sample sizes and thus inadequate statistical power make it hard to interpret the earlier findings. In addition a growing number of studies using more rigorous methods have reported null-findings regarding facial masculinity (Jones et al., 2018; Marcinkowska et al., 2016, 2018), masculine behavior (Stern et al., 2020) and vocal masculinity (Jünger et al., 2018). Also, two meta-analyses have been conducted using largely overlapping studies. One found positive results for preference shift reporting a small effect size (Gildersleeve, Haselton, & Fales, 2014), and the other did not find support for dual mating strategy (Wood & Carden, 2014) adding up to the controversial results.

There is also ambiguity in research regarding hormonal contraceptives. Two within subject studies have been conducted, first reporting that hormonal contraceptive users prefer more feminine faces, and hormonal contraceptive users partners have more feminine faces in comparison with normally ovulating women (Little, Burriss, Petrie, Jones, & Roberts, 2013). However, the later and larger, study failed to replicate the earlier results (Jones et al., 2018). Also, study conducted by Jones et. al. (2018) showed that taking "placebo" (inactive pills containing no hormones) did not affect the preference. Even though dual mating strategy hypothesis has been widely accepted and studied, in the light of the recent findings it seems that the evidence it has might be due to false positives, underpowered studies and publication bias.

1.4. Current study

Aim of the current study is to test dual mating strategy hypothesis using two potential markers of genetic quality, facial masculinity and putative chemosignal androstadienone using placebo controlled, double-blind between subjects design with larger sample size (81) in comparison with earlier studies reporting significant results. First, we investigate if women in fertile phase of the cycle show increased preference for facial masculinity compared to women in non-fertile phase, as dual mating strategy suggests. Also we test if hormonal contraceptive users would differ from either fertile or non-fertile group as earlier studies have yielded contradictory results (Jones et al., 2018; Little et al., 2013).

Secondly, we test if androstadienone alters women's evaluations for male facial attractiveness depending on fertility status. If androstadienone is a signal of genetic quality, according dual mating hypothesis women should prefer it during fertile phase of the cycle as by preferring the cue women would also prefer males with high genetic quality, that emit the signal. One possible way for this is if exposure to androstadienone increases women's perceived attractiveness of male faces as shown in earlier studies (Ferdenzi et al., 2016; Saxton et al., 2008). Also, in accordance with earlier studies regarding hormonal contraceptive use, we hypothesize that women using hormonal contraceptives show decreased preference for masculinity in

comparison with groups not using hormonal contraceptives (for a review on contaceptives effect on mate choice, see Alvergne & Lummaa, 2010).

Thirdly, as earlier studies using male chemosignals (Ferdenzi et al., 2016; Thorne et al., 2002) have yielded mixed results regarding menstrual cycle and reported effect that is not dependent on menstrual cycle. To address this controversy, we test if androstadienone increases perceived attractiveness of male faces, not dependent on menstrual cycle.

Lastly, we investigate if androstadienone would enhance the preference for facial masculinity. As both, androstadienone and facial masculinity are thought to signal genetic quality either via immunocompetence or enhanced intra-sexual competition, a male with masculine face should also have more derivatives of testosterone in his body odor and vice versa. Hence, we hypothesize that androstadienone would increase the female preference for facial masculinity.

2.METHODS

2.1. Participants

Total of 82 heterosexual female Finnish students participated in the study (mean age 23.17, range 19-35) differing in fertility status. Participants were recruited via university email lists and word of mouth. Of the 82 participants one was excluded from the analysis, as she did not fill the first questionnaire regarding masculinity preference. Of the remaining participants 17 were in the fertile phase of their cycle, 30 were in the non-fertile phase and 34 were using hormonal contraceptives, including combined oral contraceptives (27), intrauterine device (3), mini pills (4). Half (41) of the participants were exposed to androstadienonone, of which 12 were in the non-fertile phase, 11 were in fertile phase and 18 used hormonal contraceptives. From the other half (40) who were in placebo condition 18 were in the non-fertile phase of menstrual cycle, 6 were in the fertile phase and 16 used hormonal contraceptives. All participants signed an informed consent form. Exclusion criteria included dysnosmia, smoking, a history of nasal trauma or brain injury as these may decrease potential effectiveness of androstadienone.

2.2. Materials

2.2.1. Androstadienone

Androstadienone was obtained from Steraloids incorporated (Newport, RI). Thirty (30) mg of crystallized androstadienone was put to a opaque jar as done in previous experiments (Huoviala & Rantala, 2013; Wyart et al., 2007) and was stored in room temperature and kept protected from light.

In order to make the androstadienone smell similar to the control substance, 30mg of yeast was mixed into androstadienone, and control stimulus contained 60 mg of dry yeast, as done earlier by Huoviala & Rantala (2013).

2.2.2. Individual pictures

Full face pictures of 40 Caucasian males were taken in controlled environment regarding the lighting and background. Pictures were then rated for attractiveness by female raters, and in that order every second picture was selected for set A and rest of the

pictures formed set B. This was done to ensure that pictures were similar in attractiveness. The order of pictures was counterbalanced so that half of the participants saw set A before and set B after administration of androstadienone or placebo and other half of participants saw the pictures in contrariwise manner.

2.2.3. Picture pairs

Picture pairs were formed from 40 full face pictures featuring Caucasian males. Full face pictures were photographed in similar fashion as described earlier. Specialist software (Tiddeman, Burt, & Perrett, 2001) was used in order to calculate the vector differences between the average male and female faces. Then 25% of these differences were either subtracted or added to the full face pictures as done in earlier studies, although the amount of morphing has varied (Jones et al., 2018; Penton-Voak & Perrett, 2000; Penton-Voak et al., 1999; Perrett et al., 1998; Lisa L. M. Welling, Jones, Debruine, Little, & Smith, 2008). Face pairs were created using feminized and masculinized version of the same face. Figure 1. demonstrates picture pairs used. Two sets of 20 pairs were formed with randomized order to rule out order effects. Individual pictures and face pairs were formed using pictures of different individuals so there was no overlap between them, and each participant saw each face only once during the experiment.

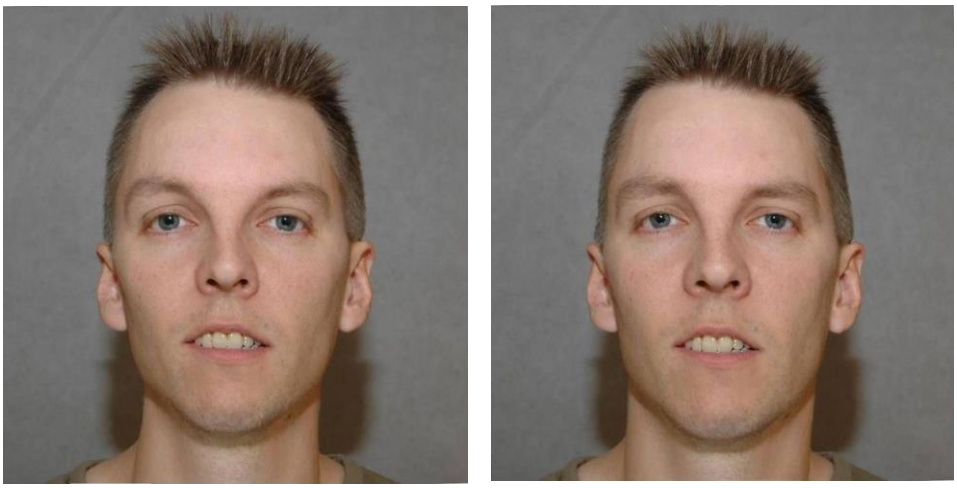

Figure 1. Example of face pairs used. On the left side face is 25% feminized from the original picture and right one is 25% more masculinized from original face.

2.3. Procedure

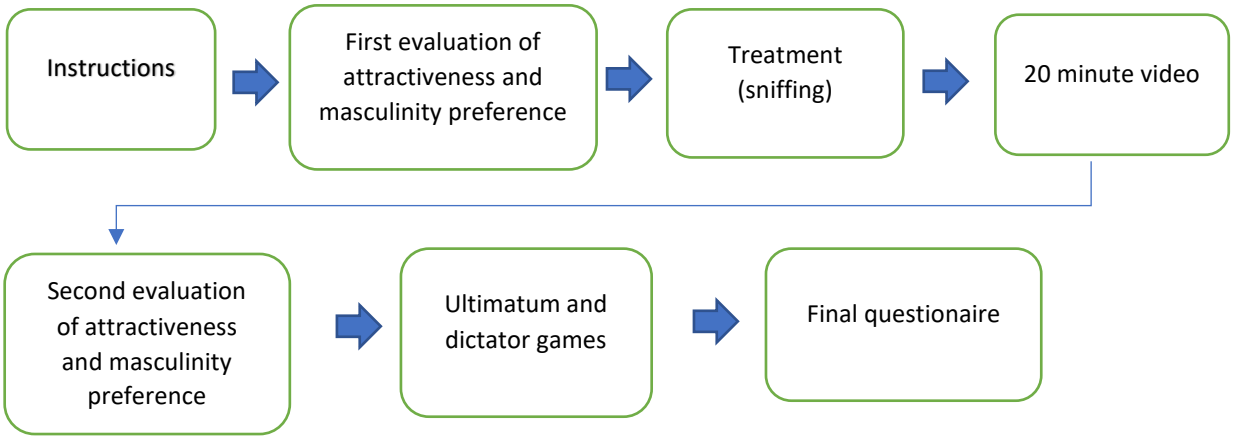

Figure 2. Procedure of the study.

Study was conducted as placebo controlled, double-blind between-subjects design. Experiment took place in an air-conditioned room, between 12:00 and 18:00 and each session lasted around 60min.

After signing the consent form, the procedure of the study was explained to the participant and also provided in writing. After confirming that the participant had understood the instructions, experimenter "turned on" the video camera, that was used to create an impression that participant is monitored throughout the experiment, although the camera was not actually on. Then the experimenter left the room for the rest of the study.

Experiment started with participants evaluating the attractiveness of 20 pictures of male faces from a laptop computer screen. Attractiveness was rated in scale from -5 (very unattractive) to 5 (very attractive). After completing ratings of individual pictures, participants were shown 20 face-pairs, consisting of different faces than the individual pictures, and participants were instructed to select the more attractive one from the pair by marking the number of that picture (1 or 2) to the answer sheet. All the pictures presented to the participant were shown in the middle of the screen using Microsoft PowerPoint. Participants had no time limit to rate the pictures and proceeded from

picture to picture by hitting space bar and provided answers to attractiveness ratings and masculinity preference with pen and paper to the answer sheet.

When participants had completed all 20 face pairs, they were instructed to open the sealed opaque jar containing either androstadienone or control and were instructed by computer program to sniff the jar 20 times for 5 second each time, taking every other sniff with right nostril and every other with left nostril, so that total of 10 sniffs were taken with left nostril and 10 with right nostril.

After being exposed to the treatment, participants watched a 20min relaxing aquatic video as done in previously by Huoviala & Rantala (2013). Aim of the video was to make sure that androstadienone had enough time to take effect. As exposure to chemosignals can be seen in hormone levels after 15min of the exposure (Cerda-Molina, Hernández-López, de la O, Chavira-Ramírez, & Mondragón-Ceballos, 2013).

After the video participants task was to rate another set of 20 pictures of individual faces for their attractiveness. Following that, 20 face-pairs (again taken from different individuals than individual pictures) were shown and again participants were to choose the more attractive one from the masculinized and feminized faces. Between the face-pairs participants were instructed to sniff the treatment solution, to keep the exposure constant.

Following the second face pair evaluation participants played two rounds of one-shot ultimatum game, both as proposer and as receiver and one round of dictator game, as proposer. This data will not be included to the analysis as it is out of the scope of this master thesis, but It is mentioned here as it affected the procedure.

After three rounds, participants filled in a short questionnaire regarding age, sexual orientation, the pleasantness and intensity of the odor stimuli, the usage of contraceptives and the current phase as well as usual length of their menstrual cycle.

2.3. Statistical analyses

IBM SPSS version 25 for Windows™ software (IBM Corp, 2017) was used in all the analyses.

2.3.1. Variables

Odor intensity and pleasantness

In order to verify that masking of the androstadienone was successful participants were asked to rate the pleasantness and intensity of the stimuli. Both variables were evaluated from 1 to 10, one meaning weak intensity or unpleasant smell and 10 meaning very strong smell or very pleasant smell.

Within subject variables

From face evaluations 4 variables were formed. Attractiveness_before was formed by measuring the average attractiveness for all the 20 pictures rated before the treatment. In similar fashion attractiveness_after treatment was the average of the ratings for all the 20 pictures after treatment.

For the masculinity preference_before and preference_after the treatment were calculated by summing all the times when participant had chosen the more masculine face and then dividing that by total amount of face pairs (20). Variable is thus a percentage of how many times masculinine face was favored over more feminine face.

Between subjects' variables

Participants provided information about the length of their menstrual cycle and the day of the last menses and fertility was calculated using assumptions that luteal phase lasts 14 days, and fertile phase does not exceed 6 days (Wilcox, 2000). The fertility variable was calculated only for normally ovulating participants. Fertility variable was created using aforementioned variables, with three levels, fertile phase, non-fertile phase and use of hormonal contraceptives.

2.3.2. Analyses of odor pleasantness and intensity

Odor pleasantness and intensity were compared between experimental and control condition to ensure that potential effects were results of the compounds and not

results of differing pleasantness or intensity. Homogeneity of variance was tested using Levene's Test of Homogeneity of Variance (odor_pleasantness p = .631, odor_intensity p = .594). Normality was tested using Kolmogorov-Smirnov test of normality and both variables failed to meet the normality assumption regarding pleasantness (androstadienone_pleasantness p = .001, control_pleasantness p < .001) and intensity (androstadienone_intensity p = .001 ;control_intensity p = .012). Intensity was perceived as relatively high and odour pleasantness was perceived as low in both groups thus the distribution is skewed in both cases. Both variables were transformed using natural logarithm, but the normality assumptions wasn't met in either pleasantness (androstadienone_pleasantness p = .025, control_pleasantness p = .006) or intensity (androstadienone_intensity p < .001 ;control_intensity p = .010) and thus a non-parametric Mann-Whitney U-test was used.

2.3.3. Analyses of attractiveness evaluations and masculinity preference

Attractiveness ratings before treatment failed to meet the assumption regarding homogeneity (Levenes test, p = .009) but attractiveness ratings after treatment met the assumption (Levenes test, p = .059). Both masculinity ratings, before and after treatment met homogeneity assumption (Masculinity rating before, Levenes test, p = .431 ; Masculinity rating after Levenes test, p = .229).

Differences in attractiveness ratings were analyzed using 2 (time) x 2 (treatment) x 3 (fertility) mixed model ANOVA. Time was within subject variable and it had had two levels, before treatment and after treatment. Treatment and fertility were between subject variables, and treatment had two levels, treatment and control, and fertility had three levels, non-fertile phase, fertile phase and hormonal contraceptive use.

Masculinity preference was also analyzed using 2 (time) x 2 (treatment) x 3 (fertility) mixed model ANOVA with the same levels as mentioned above.

In order to minimize the number of hypotheses tested and to avoid false positives, only those comparisons that test aforementioned hypotheses are reported. Further as running multiple variance analyses increases the risk of a false positive and a potential consequence of failing to meet the homogeneity assumption is that the observed p-

value may decrease alpha level .01 was used instead of .05 while interpreting results from both ANOVAs.

3. RESULTS

3.1. Odor masking

Odors did not differ in terms of perceived pleasantness (U=746.5, p = .479, d = .16) or intensity (U = 724.0 p = .359, d = .20). As there were no differences in perceived odor pleasantness or intensity, potential effects of androstadienone are unlikely to be results of differing qualities of odors. Cohens d was calculated using calculator 11 in psychometrica.de.

3.2. Between group differences in perceived attractiveness and masculinity preference

Mixed model of variance analysis was conducted for both attractiveness and masculinity preference.

3.2.1. Attractiveness ratings

Analysis showed no interaction between time and treatment $F(1,75) = .11$, $p = .739$, $\eta_p^2 = .001$, or main effect for fertility, $F(2,75) = .86$, $p = .428$, $\eta_p^2 = .02$. Also no interaction between time, treatment and fertility was found $F(2,75) = .28$, $p = .760$, $\eta_p^2 = .01$. Results are illustrated in Table 1.

3.2.2. Masculinity preference

Analysis revealed no interaction for time and treatment, $F(1,75) = 2.67$, $p = .107$, $\eta_p^2 = .03$ and no main effect was found for fertility $F(2,75) = 2.94$, $p = .059$, $\eta_p^2 = .07$
Also no interaction was found between time, treatment and fertility, $F(2,75) = 2.11$, $p = .128$, $\eta_p^2 = .05$. Results are illustrated in Table 1.

Table 1. Descriptive statistics of attractiveness ratings and masculinity preference before and after treatment.

	Fertility					
	Low		High		Contraceptives	
	Mean	SD	Mean	SD	Mean	SD
	Attractiveness ratings					
Androstadienone group before	-2.00 [-2.69, -1.31]	1.21	-1.49 [-2.15, -.83]	1.11	-1.72 [-2.02, -1.42]	.66
Androstadienone group after	-1.95 [-2.6, -1.3]	1.15	-1.35 [-1.86, -.84]	.84	-1.50 [-1.93, -1.07]	.94
Control group before	-1.26 [-1.87, -.65]	1.33	-1.34 [-1.97, -.71]	.79	-1.68 [-1.97, -1.39]	.59
Control group after	-1.21 [-1.84, -.55]	1.46	-1.48 [-2.72, - .24]	1.55	-1.36 [-1.80, -.96]	.85
	Masculinity preference (%)					
Androstadienone group before	55.4 [46.9, 64.0]	16.5	59.1 [52.5, 66,0]	13.6	59.1 [52.5, 66,0]	3.81
Androstadienone group after	46.7 [39.6, 53.7]	12.5	58.7 [43.6, 73.7]	25.5	58.7 [43.6, 73.7]	3.46
Control group before	61.1 [53.0, 69.5]	16.5	30.0 [15.4, 44.3]	17.9	30.0 [15.4, 44.3]	2.68
Control group after	66.4 [56.6, 76.0]	21.2	40.0 [29.9, 50.1]	12.7	40.0 [29.9, 50.1]	3.55

Confidence interval reported is 95%
Masculinity preference means are reported as %

4. DISCUSSION

Firstly, in current study we did not find preference shift regarding facial masculinity between menstrual cycle phases, despite using larger sample size than some of the earlier studies, which is in line with recent studies also reporting non-significant results (Jones et al., 2018; Marcinkowska et al., 2016, 2018). Also, in current study no evidence was found that hormonal contraceptive use would decrease masculinity preference as group using hormonal contraceptives did not differ from normally ovulating groups regarding preference for facial masculinity. Although many earlier studies have reported decreased preference for masculinity in hormonal contraceptive users (Feinberg et al., 2008; Little et al., 2002; Penton-Voak et al., 1999), there are contradicting results (Cobey et al., 2015; Jones et al., 2018). Most notably in a within-subject study by Jones et al. (2018) negative effect was found, contradicting the idea that hormonal contraceptive use would decrease masculinity preference. If the effect were robust, contradicting results (especially in well conducted study using large sample size) would be unlikely. This suggest that either the effect is not robust, or that there is no effect. Result of current study did not find support for the idea that hormonal contraceptive use decreases masculinity preference and as there are contradicting results on the matter studies using larger sample sizes and within-subject design are needed to resolve the controversy.

Secondly, in current study androstadienone did not affect attractivity evaluations depending on phase of menstrual cycle or use of hormonal contraceptives as dual mating strategy predicts. Earlier studies (Ferdenzi et al., 2016; Thorne et al., 2002) have also reported no clear effect between fertility and androstadienone, and to this date there is no clear evidence that androstadienones effects are dependent on fertility. Also, no main effect was found for androstadienone regarding facial attractiveness as we hypothesized, adding up to the findings of Hare et. al. (2017). In current study 30mg of androstadienone was used, which is larger amount than typically found in male axillae (Gower & Ruparelia, 1993), and its it unlikely that null findings are due to a weak signal. To this date 2 out of 4 studies (including current one) report significant findings (Ferdenzi et al., 2016; Saxton et al., 2008), and study

conducted by Saxton et al. studied androstadienone´s effect in speed dating, instead of laboratory settings and is thus more susceptible to confounding variables. It Is possible that androstadienone increases perceived attractiveness, but studies investigating the issue with small sample sizes may have been underpowered and therefore it is hard to make correct statistical inferences based on them.

Lastly, no main effect was found androstadienone regarding facial masculinity. This part of the study was entirely novel, and rationale was that androstadienone would increase preference for facial masculinity, as these two traits can be expected to appear together. On the grounds of this study there is no compelling evidence that androstadieonone would alter preference for masculinity.

It is of special importance to point out that current study's (and of those published earlier) results regarding androstadienone are tricky to interpret as three possible explanations arise. One is that androstadienone does not signal genetic quality, and nothing can be said about dual mating strategy. Second one is that androstadienone does signal genetic quality and the results do not support dual mating theory. Last possible explanation is that there is interaction between androstadienone and menstrual cycle, but studies have not been able to find the effect. Evidence for androstadienones role as chemosignal is scarce as only 4 studies (including current study) have been conducted regarding intra-sexual competition and half of these studies have failed to find the effect (andostadienone is discussed in greater extend below). Dual mating theory has also met recent critique (for a review, see Jones et al., 2019) and thus it is hard to interpret the results as neither, androstadienone or dual mating strategy theory, seem to have strong support behind them.

There has also been critique for use of synthetic chemosignals (or pheromones), most notably no peer reviewed bioassay has been reported where androstadienone (instead of any other compound found in axillae) acts as chemosignal (Wyatt, 2015). For a more comprehensive critique, see review by Wyatt (2015). One study has found that chemosignals collected from male axillae positively altered female evaluations of male faces (Thorne et al., 2002). It is possible that chemosignals collected from axillae may alter the evaluations of attractiveness, but, once again, more studies are needed in that matter. Current study failed to find effect for androstadienone, and in the future a

larger study using within-subjects paradigm should be conducted to address whether androstadienone plays a role in human chemosignaling.

Recently growing number of studies have failed to find support for dual mating strategy hypothesis. In current study no support for dual mating hypothesis was found, albeit two different signals of genetic quality were used, and a larger sample was collected. These findings are in line with recent studies, that have used within subject -paradigm and larger sample sizes. In a comprehensive review Jones et. al. (2019) have pointed out methodological problems in studies reporting significant results regarding dual mating strategy hypothesis, mainly low statistical power due small sample size and use of between subject -design. As the statistical power of earlier studies has been low, it is probable that significant results are due false positives which is supported by larger studies failing to replicate these earlier results (Jones et al., 2018; Jünger et al., 2018; Marcinkowska et al., 2016, 2018; Stern et al., 2020). Thus, although dual mating strategy has been influential in human mating psychology it does not have strong empirical support.

As suggested by Jones et al. (2019) in the future studies should test dual mating hypothesis against alternative hypothesis such as "estrous" model (Havliček, Cobey, Barrett, Klapilová, & Roberts, 2015) as currently there is not clear evidence for dual mating strategy, and studies reporting negative effects, and thus contradicting dual mating strategy, are scarce (Harris, 2013; Zietsch, Lee, Sherlock, & Jern, 2015). Estrous model suggests that instead of change in masculinity preference, near ovulation interest in sex would be greater. Thus, according to "estrous model" there is no adaption for obtaining good genes while maintaining support from another individual, but rather a by-product of shared ancestry with other primates that have increased sexual motivation during estrous.

4.1. Limitations of the study

Most notable limitation of the current study is self-reporting of menstrual cycle. As noted in Gangestad et al. (2016) calculating timing of ovulation from last menses is not accurate measurement of ovulation. Length of both follicular and luteal phase vary

among women, and even though fertile phase was calculated using 6 day fertile window, Gangestad et al. (2016) estimate methods validity to be notably lower compared to other techniques, such as confirming the day of menses after the experiment, or by using hormonal test. As the validity is low, chance that there are participants who are not in fertile phase of the cycle in the fertile group is higher, as is the chance that someone in her fertile phase was in the non-fertile group. Hence using self-reported timing of ovulation can decrease the differences between fertile and non-fertile groups and lower statistical power of the study.

Also using estimating timing of ovulation results in uneven group sizes. If all participants had regular cycles, using 6-day fertile window means that every fifth non-contraceptive user would be in fertile phase. As not every woman has a 28-day cycle, and use of hormonal contraceptives is common, the fertile group ends up significantly smaller. In addition, if fertile group is split in half (treatment/control) this halves the already small group size. The fertile group in placebo condition had only 6 participants, which lowers the statistical power in the current study.

Another limitation is the use of between-subjects design. To observe effect size of .5 with 80% power would require sample size over 900 participants with between subject design while controlling for potential error in evaluating phase of cycle (Gangestad et al., 2016). As shown in meta-analysis by Gildersleeve et al. (2014), effect size of preference shift towards masculinity might be closer to small (.2) than medium (.05). Thus, the current study's probability to find a possible effect with 81 participants is low. For comparison Gonzalez and Ferrer (2016) estimated that sample size of 200 has statistical power of 20% to yield positive results if effect size is .05/medium. On the grounds of power analysis by Gangestad et al. (2016) and Gonzalez and Ferrer (2016), using between subjects -design decreases the statistical power significantly. At the time of collecting the data for the current study (2012) this issue was not widely known. Power analyses before data collection should be done in future studies.

There are also some methodological aspects that should be considered while interpreting results. Evaluations of attractiveness and masculinity preference before and after the treatment were not done in identical settings, as participants were asked to sniff the jar between the pictures. This can be a confounding variable on current

study. Secondly the use of term attractive may have been ambiguous. As context (choosing short- and long-term partners) has been reported to potentially have an effect on preference (Penton-Voak et al., 1999) we cannot rule out the possibility that participants understood the term attractiveness differently. Some participants may have evaluated individual pictures thinking of suitability to short-term relationship, whereas others may have thought of attractiveness in long-term relationship. Another problem arises from use of only two pictures while evaluating masculinity preference. Although this method has been used in many earlier studies (Cobey et al., 2015; Penton-Voak et al., 1999; Perrett et al., 1998; L. L.M. Welling et al., 2007) it doesn't address the individual variation. If a participant prefers more masculine faces overall, it is possible that in both phases, fertile and non-fertile, she prefers the more masculine picture in forced choice task, possibly weakening the statistical power in current study. Thus, we cannot conclude that tasks used in current study may not be reliable measurements of attractiveness and masculinity preference.

Although facial characteristics are important factor in mate choice and have been studied widely (Johnston et al., 2001; Law Smith et al., 2006; Perrett et al., 1998), linking these characteristics to evolutionary important traits has resulted in contradictory findings. Male facial dimorphism (Rhodes et al., 2003; Thornhill & Gangestad, 2006) and attractiveness (Foo et al., 2017) have been found to correlate with some aspects of health, such as mental health (Rhodes, 2006), sperm quality (Foo et al., 2017), but most studies have relied upon questionnaires and validity of health measurements is questionable. Only study that used measures theoretically linked to sexual selection reported a weak link between facial attractiveness (predicted by facial dimorphism) and semen quality (Foo et al., 2017). Also male sex hormones have failed to show immunosuppressive properties (Nowak et al., 2018), questioning the link between testosterone dependent trait´s role as honest signal as well as potential link between facial dimorphism and health. Hence it is possible that male face dimorphism, as female face dimorphism, signals fertility instead of overall health. It has also been suggested that masculinity is preferred not because of its connection to health but rather because its connection to dominance (Boothroyd et al., 2007). All in all, there is

no consensus why masculinity would be preferred nor is there strong evidence that masculinity signals health or fertility.

Also, in current study amount of androstadienone was notably larger compared to naturally occurring amounts. One might criticize that effects found using amounts that exceed those occurring naturally tell nothing about real life effects of androstadienone. While this is true, as no effect was found, it can be interpreted that if androstadienone would act as signal, larger amount should elicit larger effect and hence using unnatural amount is not a problem while interpreting current results.

Last limitation regards the sample of the current study. As normal population does not constitute of university students, our sample does not represent the whole population. In addition, current study had a convenience sample, as some of the participants were recruited via word of mouth. It is possible that some participants recruited by word of mouth were more prone to please the experimenter, for example by knowing the experimenter. No significant findings were found, so potential misrepresentation of population does not interfere with interpretation of results.

4.2. Conclusion

In conclusion, no support was found for either dual mating theory or androstadienone´s role as human chemosignal of genetic quality. Although current study may be underpowered regarding dual mating theory, at the time of collection of the data, methods used were common in this field of study and current had larger sample size than many of its predecessors. It has more recently been shown that earlier studies employed methods that were unlikely to have adequate statistical power, and studies done in the future should use larger sample size and employ within-subjects paradigm and more objective evaluation of menstrual cycle phase. Current findings regarding dual mating hypothesis are in line with recent studies. In addition, no evidence was found for androstadienone`s role as chemosignal of genetic quality. We suggest future research to be directed towards potential alternatives of dual mating theory, such as estrous model. Regarding androstadienone, as the findings, seem ambiguous we suggest that following studies should address the issue

with larger sample size to resolve the ambiguity around androstadienone and to clarify if it actually acts as a human chemosignal.

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