Human Leukocyte Antigen' influence on sexual selection and human pair bonding

Author: Silke Jansma s3865975
Date: 23-06-2021
Supervisor: Prof. Dr. J.C. Billeter, department of Biology, Genetics and Behavioural Sciences

Summary

The Human Leukocyte Antigen region contains immunological genes and has been hypothesized to play a role in sexual selection and pair bonding. Papers on how one's HLA genes affect odour preference and attraction were reviewed, with results showing preference towards dissimilar and diverse HLA profiles respectively. Psychological surveys in couples showed how similar HLA alleles between partners affect women's attraction and sexual attraction negatively, and increased the likelihood to be attracted to other men and even cheat. Lastly, the chance of fertilization and success of pregnancies based on HLA genes was analyzed. Interaction between follicular fluid and sperm appeared to be dependent on HLA, with HLA dissimilarity increasing the chance of fertilization. Furthermore, the probability of loss of pregnancy depends on certain HLA allele (combinations). All in all, pre- and-post sexual selection as well as pair bonding are influenced via the HLA.

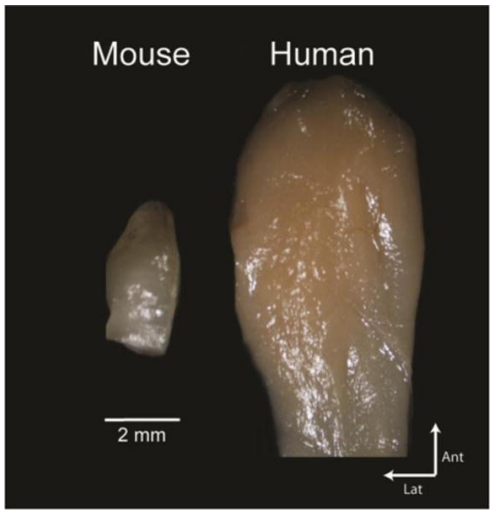
Table of content

Introduction

Being selective when exerting mate choice increases offspring's viability and attractiveness and is dubbed sexual selection. The traits on which this mate preference is based differ in importance between species, sex, and individuals. Amongst mammals, visual, auditory and olfactory signals, as well as cues indicating parental care or provision of resources play a role. It appears that the MHC-region, a part of the genome containing highly variable immunological information, affects body odour and through this contributes to (female's) preference for pathogen resistant partners (Hillgarth, 1990; Jacob et al., 2002; Esjmond et al., 2014). How MHC affects smell is relatively unclear, in animals the MHC glycoproteins are detected in bodily fluids, and a relationship with the skin's microbiome is also thought to be involved in the production of body odours (Wobst et al., 1998; Singh et al., 1990). The diversity of MHC alleles within a population depends not only on heterozygous advantage or encountered pathogens in the evolutionary history, but also on sexual selection maintaining heterozygosity via dissortative selection (Wintersnitz et al., 2013; Esjmond et al., 2014). Disassortative selection or negative assortative selection refers to a mating pattern in which phenotypically different individuals mate with each other more than would be expected under random mating. The earliest findings on MHC-based mating preference through olfaction concerned house mice (Yamazaki et al., 1976).

In humans, the MHC region is called the Human Leukocyte Antigen or HLA and is one of the most polymorphic regions of the genome, with approximately 9000 known alleles of class I, and 3000 alleles of the class II genes (Milinski, 2003). Which is one of the reasons why It's complicated to study HLA-based mate preference in people (Parham & Ohta, 1996). It is commonly believed that olfaction has become weak in humans, based on an old myth that to evolve free will, some brain regions had to shrink. Over generations the human cortex increased in size, but relative nor absolute size of the human olfactory bulb is small (Figure 1). Additionally, the number of neurons and distinguishable odours is comparable to other mammals (McGann, 2017). Several studies have shown that women prefer the smell of those men with dissimilar HLA-genotypes (Wedekind et al., 1995; Jacob et al., 2002; Santos et al., 2005). It makes sense that women, being the choosier sex based on the limited amount of producable offspring, are more aware and selective for HLA based preferences than men. The hypotheses Penn and Potts constructed for women's HLA preference include that HLA-heterozygous offspring have increased immunocompetence, and that HLA disassortative mating preference lowers incest rates (Penn & Pots 1999). The preference for HLA heterozygous faces is independent from full genome heterozygote preference, meaning that it's less likely to be an incest avoidance effect (lie et al., 2008). Mcclelland, Penn and Pots showed in 2003 that individual heterozygosity at HLA loci created more resistance to infection and had higher fitness as most protective genes were co-dominant (Mcclelland, Penn Pots, 2003; Havlíček & Roberts, 2009). Still, whether selection is for maximal heterozygosity, some intermediate degree of heterozygosity or only for specific HLA genes is unclear. For example Jacob and colleagues found that females' odour preference was based on paternally not maternally inherited genes (Jacob et al., 2002). Whereas Hakkarainen found only weak effects of HLA heterozygosity on attraction unless a specific HLA locus for autoimmunity and viral infections was considered (Hakkarainen et al., 2021).

Figure 1: *Size of mice and human olfactory bulb compared (McGann, 2017)*



One could argue that partner selection with the goal of procreation cannot be compared to these singular smell preference findings. In the human situation the decision to procreate with someone is generally made on a lengthier timescale and based on many factors besides olfaction. The evidence on the strength or mere existence of the HLA-based preference amongst humans is controversial, but since humans don't often have children based on a whiff in a single meeting, different questions should be asked. For example, is there an effect of HLA dissimilarity on eventual procreation? Would a smell-preference therefore not exist in those not seeking a partner for procreation? Would the relationships between HLA dissimilar people be more successful compared to couples with more similar HLA genes, by standing the test of time or by increased satisfaction of the intimate relationship? Is there evidence for other pre-copulatory or even post-copulatory selection based on HLA genes as well? And what is the role of hormonal contraceptives, affecting fertility status, on the preference for HLA-heterozygosity in women? Is success of pregnancies dependent on variability in HLA genes between mother, father or foetus? The answers to these questions will be given based on multidisciplinary research from fields of immunology, genetics, psychology and neuroscience. This paper first discusses relevant preliminary research on how odour perception is affected by HLA, both the oldest, and the latest papers. After which some articles comparing existing couples are considered. Later, the questions regarding the success of fertilization and loss of pregnancy are talked about. Hopefully a general consensus can be made on whether HLA-based preference actually shapes human generations.

Results

HLA affected odour preference

As immune system influenced odour preference is found in many species (Yamazaki et al., 1976), it was expected to play a role in humans as well. The first paper attempting to discover such a smell preference in humans used T-shirts worn by men at night for two days and scored by women with known use of hormonal birth-control (Wedekind et al., 1995). The degree of HLA similarity was based on the genotypes of three HLA genes: A, B and DR, and each participant assigned a pleasantness score to six samples. With the result revealing that the women not on birth control considered the smell of males with dissimilar HLA alleles on these loci as slightly more pleasant. Interestingly an insignificant preference for similar HLA genotypes was found for women taking hormonal anticonception, more will be told about this later. Although the sample size was small and only three genes were taken into account, this paper created a snowball effect of odour dependent sexual selection research.

Two years later, Wedekind and Füri performed a follow-up study that looked into both men's and women's odour preference this time. With a similar set-up, a significant preference for HLA dissimilarity was found only when looking at the sample as a whole, including men and women. Furthermore, those with preference for HLA dissimilar samples reported that the smells were reminiscent of current or past partners, indicating that mate preference is influenced by HLA-based olfactory preference (Wedekind & Füri 1997). More support for HLA disassortative mate preference amongst men is limited, another study did find that men had an odour preference for women during the fertile phase, which wasn't perceived in females using oral contraceptive pills (Kuukasjarvi et al., 2004). Men's odour preference being at least partly based on women's fertility indicates men's preference for non HLA-based odours, complicating odour preference research further. Another paper on facial and odour preferences influenced by the HLA supports men's odour preference for HLA dissimilar women and those rated as more facially attractive. Yet surprisingly women's odour preference was unrelated to HLA dissimilarity, instead more heterozygous males were favoured (Thornhill et al., 2003).

A more recent study on the HLA based odour preference used a different approach. Female and male Brazilian students provided sweat and urine samples and were phenotyped for the HLA-A and HLA-B loci. In this research, sweat was collected via a cotton ball touching the chest area. Participants had specific instructions, like not eating strong smelling food, to limit confounding variables. No difference between birth-control or naturally cycling women was observed and men were overall less sensitive to smells than women. Participants had increased difficulty categorizing a smell as pleasant, indifferent or unpleasant with higher similarity in HLA alleles with the samples. Of everything investigated, only women significantly preferred the smell of dissimilar HLA males' sweat, men had no significant preferences nor did anyone have preferences for the urine samples (Santos et al., 2005).

Another paper worth discussing was aimed to answer whether an odour preference for unsimilar HLA types was due to childhood exposure creating non-sexual associations based on experience or based on inherited HLA alleles. Genetic information of five HLA loci was gathered from female participants, male odour donors and participants' parents. On those loci, a maximum of ten alleles could thus be shared between judger and donor of smell samples, the actual difference within the sample was between 0-7 allele matches with an average of two allele matches. Odour of donors was again based on worn T-shirts, and the participants assigned scores based on familiarity, intensity, pleasantness and spiciness without knowing where the smells came from. Surprisingly, the HLA-associated odour preference was determined only on paternally inherited HLA alleles. Results showed that the most preferred odour had an average of 1.4 alleles in common, whilst the least preferred odour had 0.6 allele matches. The conclusion seems unaligned with previous studies that show preference for the most heterozygous HLA genotype but it is not. Previous studies found a preference for more heterozygous mates when given the choice between very heterozygous or more homozygous samples (Thornhill et al., 2003). However, this paper investigated the preference within a low range heterozygous population and found a preference for a small, intermediate amount of heterozygosity. Thus it showed support for the theory that odour preference is based upon an optimum of intermediate heterozygosity (Jacob et al., 2002).

HLA genes' effects on olfaction

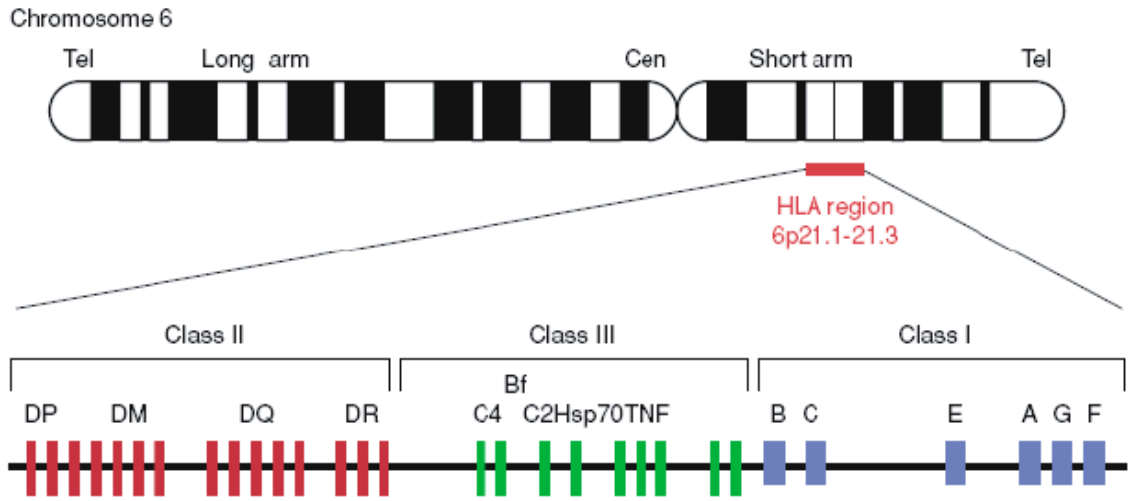
HLA genes are found on chromosome 6 and encode peptide antigens presented to T-cells that are central to the adaptive immune response (Figure 2). There are three types of HLA genes, two are of interest for this discussion: class I and class II. Class I is expressed by all somatic cells, comprises amongst others the HLA-A, -B, -C genes and mostly binds pathogen derived intracellular peptides. Class II genes are expressed by antigen presenting immune cells such as T-cells and natural killer cells, include the genes HLA-DQB1, -DQA1, -DR, -DRB1 and binds extracellular parasite peptides (Milinski, 2003; Klein & Sato, 2000b). Class I genes are thought to impact body odour the most as gene products are produced by most cells in the body. Body odour is released via glands in the skin, and personal differences in number and activity of glands, body hair amplifying odour, skin microbiome and personal hygiene affect the distinct odour given off (Kreyden, Böni, Burg, 2002). The odours perceived and excreted by humans are volatile, or evaporating at body temperature, and many factors impact perception by others. In other mammals MHC genes affect one's personal odour as the glycoproteins transcripts are found in various bodily fluids like saliva and sweat. The peptides serving as Class I ligand molecules activate olfactory and vomeronasal neurons (Leinders-Zufall et al., 2004). In vertebrates, the VNO is used in pheromone communication. However, only about a third of the adult human population seems to have this organ, with higher numbers found in children and newborns. Also, whether human VNO is functional remains an unanswered question as the ion-channels involved in signal transduction appear non-functional due to mutation. Yet electrical stimulation does show functionality of receptors (Stoyanov et al., 2018).

So far, evidence linking chemical odour compounds to HLA loci is lacking, one theory is that the HLA derived peptides act directly as odorants, but the chemical nature of the peptides make this

implausible (Natsch & Emter, 2020). Odourless secretions in skin glands are changed into detectable volatile peptides by bacteria of the skin, and could therefore be the underlying mechanism of HLA genes' effect on personal odour. For example Corynebacteria, more found on men's skin, produce pungent smelling carboxylic acids and androgen steroids (Ferdenzi et al., 2020). Even though the exact mechanism is unknown, HLA peptides are detectable by humans, and show activation of the medial frontal cortex. The difference between self and non-self HLA-based odours is distinguishable even without the VNO (Milinski et al., 2013).

Olfactory receptors' (OR) function is to perceive odours and are found somewhat clustered throughout the whole genome. Interestingly, all vertebrates have an evolutionary conserved cluster of OR genes physically close to the MHC class I region. Younger and colleagues sequenced 36 human OR loci that are linked to the HLA (Younger et al., 2001). Structural analysis between human and mice OR loci showed that the clusters were evolutionarily conserved. These HLA-linked ORs are thought to be responsible for differences in odour preference (Tizard & Skow, 2021).

Figure 2: *The HLA Loci on Chromosome 6 (Berlingerio et al,. 2009)*



HLA related perfume preference

Perfumes are connected to sexual communication, it is used to mask unpleasant body odours, stimulate (sexual) memories. The following paper written by Milinski and Wedekind considers if one's HLA profile is of importance for preference of certain traditional perfume ingredients, which would mean perfume choice is a direct sexual signal based on immunocompetence. Participants came partly from past research performed by Wedekind and were typed for HLA-A, -B and -DR loci. The first experiment was conducted to uncover if relative scoring of 36 ingredients for self was dependent on the HLA alleles of the judger. The second experiment consisted of two vessels of 18 of the 36 ingredients and the participants rated on preference for self or in another. The outcome indicated that those with similar HLA alleles had high preference

for some of the same ingredients for one's self. The two tests were taken two weeks apart, and had some of the ingredients in common. The results on the 18 smells for self were a replication of the first experiment, thus confirming a consistent preference for certain perfume ingredients to wear. No significant odour preference for another was found. The theory that perfume is chosen to amplify one's HLA type as a sexual communication is hereby supported (Millinski & Wedekind, 2001).
Another research with the aim to sell personalized HLA-based perfume confirmed Millinski and Wedekind's findings on the same 3 alleles. Odours were scored by participants, and researchers looked for preference correlations between HLA types. Only common alleles showed the same preference for certain parfume ingredients, probably due to statistical strength, and a repetition 2 weeks later showed that these results were consistent once more (Hämmerli, Schweisgut, Kaegi, 2012).

Importance of olfaction in sexuality

Sexual orientation has an established correlation with olfaction, for example homosexual men are more sensitive to the smell of androsterone than heterosexual men (Lübke, Schaslitzky, Pause, 2009). To understand if olfactory preference is a tool to find a partner for healthy children, which would not be the case amongst homosexuals, White and Cunningham aimed to discover if olfaction is more important amongst heterosexuals, compared to homosexuals.This was done by comparing the importance of olfaction in attraction for homosexual men or heterosexual men and women with a romantic interest survey. No difference in self assessed importance of olfaction between the sexes was found. Homosexual men valued olfaction less when considerering a potential partner, instead valued aspects like sound of voice more (White & Cunningham, 2017). To confirm the function of olfactory preference is healthy progeny, further research needs to be performed. A paper on degree of HLA similarity between homosexual and heterosexual couples could provide more answers for example.

HLA affecting visual attractiveness

Besides evidence on HLA affecting attractiveness of body odour discussed above, the HLA region also appears influential on facial attractiveness. Studies have found mixed results: a paper concluded that facial attractiveness is increased with HLA similarity (Havlíček & Roberts, 2009). Additionally, HLA similar men were (insignificantly) preferred by women based on facial attraction and quality of skin (Roberts et al., 2005). Whereas Thornhill and colleagues concluded odour preference for those women considered as facially attractive (Thornhill et al, 2003). Preference for HLA heterozygosity specifically, not genome wide heterozygosity, in men's faces was found by Lie and colleagues (Lie et al., 2008). On average, mixed-race individuals, with higher assumed (general) heterozygosity, have been rated as more attractive than single races (Lewis, 2010). Methods differ within facial attractiveness studies based on categorization of HLA similarity, included loci and diversity of participants.
One of the more recent studies looked at HLA's influence on facial and bodily attractiveness. Pictures of participants were scored, and genetic information was collected on several HLA genes. The results showed a preference for general HLA-heterozygous faces of both sexes.

After controlling for multiple testing and considering adiposity and age, the effect became insignificant. Notably, when separate loci were focussed on, heterozygosity of the class II DQB1 gene, involved in autoimmunity and viral infections, did show a significant effect in facial attraction (Hakkarainen et al., 2021).

Effect of oral anticonceptives on women's olfaction

Many papers have reported a disruptive effect of contraceptives on odour preference amongst women (Wedekind et al., 1995; Allen et al., 2019; Roberts et al., 2008; Millinski & Wedekind, 2001), whereas another did not (Santos et al., 2005). A study aimed to put an end to the discussion tested women before and after initiation of hormonal birth control, and compared results with a control of non-users (Roberts et al., 2008). Phase of the menstrual cycle did not affect ratings between HLA similar or non similar men; nor did use of the pill. Relationship duration did influence men's attractiveness ratings of women positively. Furthermore, a relative shift towards HLA similar preference after birth-control initiation was found, suggesting that oral birth control does disrupt odour-mediated disassortative selection.

Several hypotheses exist regarding the effect of oral contraceptives on odour preference. One states that the pill induces pregnancy signals, changing fertility status. The hormones included in hormonal anticonception are progesterone and estrogen. During pregnancy, levels of these and other hormones vary and the pill does not mimic this exactly. Another hypothesis is that birth control affects behavioral variables, such as how relationship status can lead to initiation or how prevention of pregnancy decreases selectivity of sexpartners (Roberts et al., 2008). A recently published paper looked into the effect of the withdrawal period of anticonceptives on ability to discriminate between body odours. Womens' ability to distinguish odours in different phases of the menstrual cycle was assessed, for naturally cycling women and those taking oral anticonceptives. Naturally cycling women were better at discriminating odours in the follicular phase, just before ovulation. It turned out that the withdrawal period of the pill indeed negatively affects the ability to discern human based but no ordinary odorants. Which would explain why odour preference findings amongst women on the pill are limited and weaker compared to naturally cycling women (Endevelt-Shapira et al., 2020).

Similarity in HLA genes on stable partner attraction

To discover whether HLA based mate selection is of significance in human pair bonding, HLA similarity of couples were compared to non-spouse pairs. The HLA region generally, nor any locus, allele or SNP was found to significantly influence couple formation (Qiao, Powell, Evans, 2018). In an older study on an isolated group of European setllers in the Americas, the Hutterites, a similar question was asked. This religious group of people tend to marry other Hutterites with the help of a marriage ceremony in which individuals can choose between a few

partners. The amount of HLA similarity was based on five HLA haplotypes, HLA-A, -B, -C, -DR and -DQ, which were known to be of limited variability between the 411 couples. Actual couple formation was compared to non-random couple formations based on for example founder assumptions. It turned out that partner choice in this isolated population was based on dissimilar HLA sexual selection (Ober et al., 1997).

Besides looking at the chance HLA-dissimilar couples end up together, in-pair quality of relationships with differences in HLA similarity is important for this discussion as well. The first paper used a questionnaire (filled in during different stages of female participants' ovulation cycle), and screening of HLA-A, -B, and -DR-beta (Garver-Apgar et al., 2006). Questions regarding cheating, past partners, sexual interest in partners or other individuals were asked. With higher HLA similarity in the couple, only women felt less attraction, arousal and not as sexually responsive. In addition, it predicted the amount of cheating or extra-pair attraction especially during the fertile phase. This result is supported by a study in which women in relationships with HLA-similar partners were more interested in HLA-dissimilar smells, presumably to increase offspring's genetic quality without losing the qualitative spouse (Roberts et al., 2008). Support for this theory has been found in birds (Freeman-Gallant et al., 2003).

The following paper investigated the effect of HLA type on partnership, sexual satisfaction, and “child-wish” in couples. The questionnaire consisted of descriptive questions regarding for example length of relationship or number of children, and satisfaction ratings on various parts of the relationship including sex life. DNA was analysed with help of Next Generation Sequencing on HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 genes. Identical alleles as well as alleles within the same G-group were considered matches. This paper revealed that immunological compatibility ,here hypothesized to be dissimilarity on all class I genes, does impact partnership-and-sexual satisfaction. Specifically higher partnership satisfaction is related to dissimilarity in both sexes, in women based on HLA-B and HLA-C loci but in men only on HLA-C. Whereas dissimilarity affects sexual satisfaction in women based on the entire HLA profile, mostly based on HLA-B. In men general dissimilarity did not enhance sexual fulfilment, yet dissimilarity based on HLA-B alone did. Women in relationships with HLA class I dissimilar men had an increased childwish, this was particularly based on HLA-C. The body odour questions revealed that HLA class I dissimilar partners had a more attractive odour (Kromer et al., 2016).

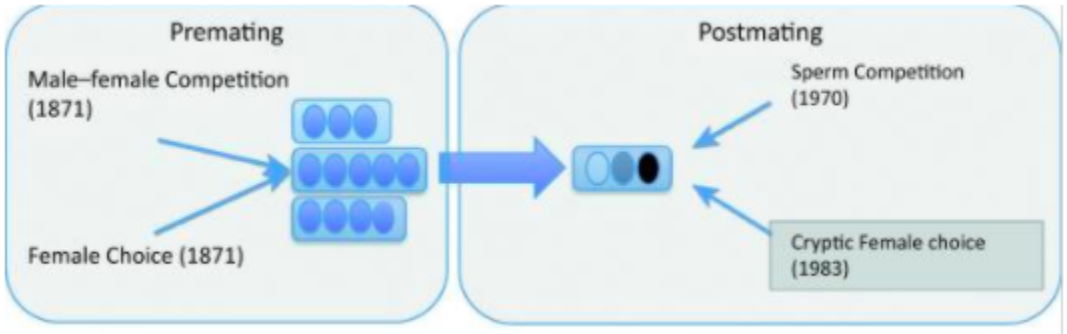
Another paper concerning couples confirmed women’s reduced sexual attraction towards the partner and increased cheating or attraction to others when there was greater allele sharing (Garver-Apgar et al., 2006). Contrary to these papers, no significant differences on overall in-pair attraction between HLA-A, -B, -DRB1 similar and dissimilar couples was confirmed (Saphire-Bernstein et al., 2017). Based on all participants, with varied ethnicities, no significant results were realized. However, when a closer look into the all-asian couples was taken, a significant effect of HLA similarity on sexual responsiveness was discovered. Overall, this finding suggests that the impact of pathogens in the evolutionary past, or ethnicity of partners, can contribute to the influence of HLA on intimate relationships.

The next paper discusses more than one question, with surprising results. Couples and fake, randomly assigned duos received ratings based on similarity of body odours, that was collected via a cotton patch worn on the armpit. Both natural samples and ones with artificially added fragrances were scored by on average 27.5 people of all ages, for resemblance between partners. The hypothesis was that selection for different odour preference in partners would result in unsimilar smells between partners. Yet partners' natural odours were found to be more similar to one another than random pairings. Additionally, an effect of birth control on similarity of body odour was researched based on if the women took any at the start of the relationship. Women taking birth control at initiation of the relationship smelled less similar to the partner than couples not on birth control, but more similar than fake couples. Perceived similarity and relationship satisfaction were also compared, with the finding that women were more satisfied in partnerships with relatively dissimilar smelling partners, the opposite appeared true for men (Allen et al., 2019).

Post-copulatory HLA-based selection

The above mentioned publications have been about preferences that influence sexual selection, or selection before copulation. In addition to the papers supporting pre-copulatory selection, evidence has been found for post-copulatory selection for HLA dissimilarity. Post copulatory selection comprises processes influencing fertilization chance of individuals during and after sex. The following paper suggests that chemoattractants released by the egg exert female cryptic post-mating choice. Meaning that based on males phenotype, non-random selection occurs within the female body, hence the cryptic nature as it's difficult to observe (Firman et al., 2017). Interaction between sperm and follicular fluid between couples and non-couples were compared. The spermatozoa accumulated repeatedly towards the same female, not necessarily the partner's. It was found to be independent of the quality or amount of chemosignals from the egg, instead appearing based upon male-female interactions; indicating female cryptic post copulatory selection (Fitzpatrick et al., 2020).

Figure 3: *Pre-copulatory and post-copulatory selection (Firman et al., 2017)*



Spermatozoa express HLA class II, to a lesser extent class I molecules, and surprisingly also HLA-linked olfactory receptors on the sperm cell surface (Sereshki et al., 2019). Follicular fluid's

biochemical composition affects fertility, and contains HLA originated molecules as well. Whether these HLA signals mediate gamete level mating preference in humans was the question the following paper discusses. The sperm of eight men was exposed to the follicular fluid of ten women. All participants were genotyped on genome wide SNP divergence and HLA similarity. Motility of the sperm, viability, movement of the tail or hyperactivation, and the pre-fertilization reaction of the sperm to fuse with the egg (acrosome reaction), of the 80 combinations were compared. Similarity of HLA genes affected sperm swimming velocity and hyperactivation negatively, but didn't have an effect on viability of sperm nor on the acrosome reaction. No effect could be established of the entire genome similarity on sperm's physiological preparation for fertilization. Thus, gamete level mate selection for dissimilarity is based on compatibility of specific HLA genes and not on the entire genome. This was an important discovery, infertility may not solely be because of pathological reasons, but be based on incompatibility of a couple instead (Jokiniemi et al., 2020).

So interaction of sperm with follicular fluid in the female reproductive tract or FRT shapes reproductive success (Fitzpatrick et al., 2020; Jokiniemi et al., 2020). The following paper tried to discern the role of HLA class I alleles and immunoglobulin (IgH) or antibody binding regions (eplets or functional epitopes) dissimilarity on gamete-level sexual selection. Different alleles can give rise to the same immunogenic properties and eplets so dissimilarity on loci might not represent compatibility. Instead, HLA similarity characterized by eplet similarity provides a possibly better mechanism of determining reproductive success. Sperm was exposed to cervical mucus besides follicular fluid. Results showed that sperm viability was increased in follicular fluid with eplet dissimilarity and in cervical mucus with immunoglobulin dissimilarity. Based on this and the above-mentioned studies, female post-copulatory cryptic choice exists amongst humans, and might be based on other genes of the female's immune system besides the class I HLA genes (Magris et al,. 2021).

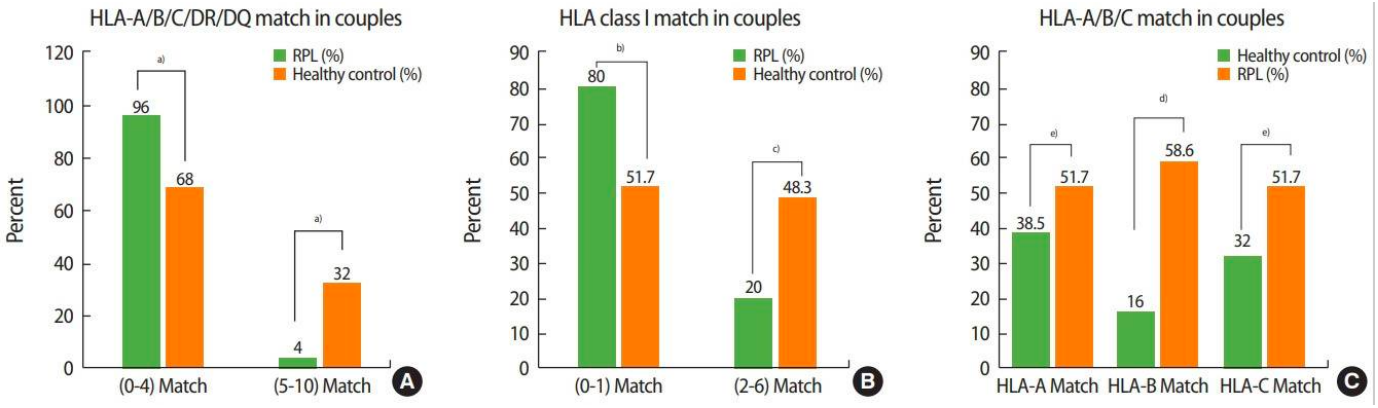
Recurrent spontaneous abortions and HLA

Two hypotheses exist to explain HLA's role in recurrent spontaneous abortions (RSA), the genetic hypothesis states that homozygosity for recessive lethal alleles that are in linkage disequiliirum with certain HLA alleles is responsible. For example, the HLA-DRB1*03 allele or physically close genes are more prevalent in women suffering from recurrent miscarriages (Kruse et al., 2004). Whereas the immunological hypothesis suggests that a sufficient immune response based on distinct paternal HLA, is needed for implantation of the embryo in the uterus. Evidence suggests that HLA similarity decreases pregnancy success, offspring's infection resistance and plays a role in some cases of RSA (Beydoun & Saftlas, 2005; Arora et al., 2020; Meuleman et al., 2015). Therefore, the HLA dependent body odour based pre-copulatory selection can be a mechanism to prevent the above mentioned phenomena. Studies on how HLA-genes affect RSA remain inconclusive, and often show an effect of certain faulty alleles or polymorphisms instead of a conclusive effect of incompatibility between parent's immune systems (Beydoun & Saftlas, 2005).

The HLA-G gene is a key candidate for playing a role in RSA as it's expressed in extravillous trophoblasts, is found in amniotic fluid and cord blood; furthermore, as HLA-G interacts with immune cells, it contributes to the formation of fetus' arteries and immune system (Xu, Zhou, Wei, 2020). One study looked into the distribution of HLA-G alleles in Danish fertile and RSA couples. The data showed that a higher frequency of G*0106 allele was found in women suffering from RSA, compared to the control. Additionally, a polymorphism in exon 8 was investigated, healthy women more often had heterozygous alleles for the polymorphism in contrast to male and females in RSA couples (Hviid et al., 2002). The opposite was found amongst chinese women, heterozygosity for the same polymorphism was found more so amongst infertile women, possibly due to ethnic differences (Xue et al., 2007).

Another study showed that the combination of a certain allele of killer immunoglobulin-like receptor, involved in recognition of HLA-C on trophoblasts, with certain HLA-C alleles influences spontaneous abortions. Heterozygosity of the HLA-C protected women from RSA with KIR-AA gene and with homozygous HLA-C2C2 partners (Nowak et al., 2011). Furthermore, a paper investigating KIR and HLA alleles between Turkish couples with and without recurrent pregnancy loss (RPL), supported the role of HLA-C2 homozygosity in men on RSA. This paper also looked into the amount of allele matches on five HLA genes between RSA parents and parents who have had successful pregnancies. No overall correlation could be found. When the couples were divided between either 0-4 allele matches or 5-10, it showed that most RSA couples had 0-4 matches indicating a need for HLA compatibility between parents (Figure Xa). When only the HLA class I genes were considered (Figure Xb), most RSA couples were found to have 0-1 allele match, the last figure showed that mismatches of HLA class I genes contributing to RSA was due to HLA-A, -B incompatibility (Figure Xc). This paper shows thus that as less matching HLA genes are more often found within couples suffering from RSA, a certain compatibility of HLA genes is necessary for successful pregnancy, contrary to the immune hypothesis (Elbaşı et al., 2020). Many papers have tried to decipher the exact role of HLA genes on RSA, and this thesis cannot discuss the enormous amount of literature available. From the abovementioned papers no overall conclusion can be drawn on specific genes. It can be concluded that RSA is linked to the HLA somehow, possibly through linkage disequilibrium with certain alleles. Future research should focus on the HLA genes of the foetus to better understand what went wrong with the pregnancy besides the incompatibility of the parents.

Figure 4: *Number of allele matches in couples with and without pregnancy complications (Elbaşı et al., 2020)*



Discussion

Understanding the subtleties of partner choice amongst humans can help with increasing quality of relationships, success of pregnancy and health of offspring. Besides evidence for odour, facial and perfume preferences based on HLA, other linked topics such as HLA related pre- and -post copulatory mechanisms and effect of HLA on relationship parameters were taken into account.
Humans, particularly women, are attracted to certain body odours, with most evidence pointing towards a preference for HLA dissimilar men but also for HLA diverse men. The papers suggesting perfume is selected to enhance one's own HLA signal support odour mediated sexual selection. Because in most cultures the use of perfume, soaps and deodorants is common, so selection based on one's natural body odour would become weak.

With differences in methods of olfactory preference research, and ethnic homogeneity of samples, variability in results did not come unexpected. Different environmental pressures could have shaped preferences for certain alleles or degrees of similarity which explains contradictory results as well. For example, body odour preference papers that had ethnically homogenous populations found results for a preference more often than papers with diverse samples (Wedekind et al., 1995; Wedekind & Füri; Santos et al., 2005; *VS*. Thornhill et al., 2003). Future studies on olfactory based sexual selection should factor in women's contraceptive use, aim to standardize odour assessment with questions besides mere preference and look within and between populations.

Some of the researchers in the field have worked together with perfume experts to create a lexicon of body odours with the goal to standardize odour assessment (Allen et al., 2018). Descriptive words such as animalic for male, sweet and milky for women were found to describe odours consistently.

As olfactory cues can also signal health and fertility status, studies on smell preference should collect women's smell during the same time in the menstrual cycle, and consider health of participants generally as well (Kuukasjarvi et al., 2004; Sarolidou et al., 2020). Alternatively, instead of collected samples, synthetic peptides could be utilized for preference testing, it would exclude some confounding variables. It would be challenging as little information is known as of yet about which HLA derived molecules are responsible for which odours (Milinski et al., 2013). Another improvement on olfactory preference research would be to include as many HLA genes as possible, when only considering three like some papers have, results can be altered based on excluded genes.

The discussed papers on HLA's impact on facial attractiveness found either preference for HLA heterozygosity or similarity. Heterozygosity preference is not fully explained by general

genome-wide preference for heterosis. Facial attractiveness is based on the level of heterozygosity of HLA genes, and as those also have higher fitness, it is an honest signal of mate quality (Lie et al., 2008).

Also of importance is the recognition that within populations of varied ethnicity, human partner choice is often based on socio-economic factors such as geographical location, ethnicity or common morals and customs. Which would result in assortative mate choice, with preference for more similar HLA genotypes as a consequence (Winternitz et al., 2017). This partly explains preference for HLA similar faces or why no correlation of HLA on couple formation was found in the study on Europeans (Qiao et al., 2018).

When considering the psychological evidence, most is in accordance with the hypothesis that HLA similarity affects pair bonding negatively. The analyses on couples showed how women especially became less sexually satisfied with and attracted to a partner with a greater amount of HLA overlap. The attraction towards men outside of the relationship and increased amount of adultery fits with the finding that odour preference of HLA-similar paired up women towards other HLA-dissimilar men. Also, both the study on the lesser degree of importance of olfaction in attraction amongst homosexuals and the finding of increased childwish of women paired up with HLA-dissimilar men supports the view that the biological function of HLA based odour preference is healthy progeny. Since homosexual women were not included in this paper, and other evidence regarded mostly female's sexual selection, future research should cover this.

The evidence on post-copulatory HLA influenced sexual selection is an interesting addition to the discussion. The cervical mucus and follicular fluid in women exerts consistent cryptic mate choice for HLA-distinct signals on sperm. It appears that the high degree of polymorphism on the HLA is partly a result of post-copulatory selection increasing chances of successful fertilization between immunologically distinct parents.

Thus the high polymorphism on HLA loci is not merely due to heterozygous advantage or parasite mediated balancing selection but also on disassortative pre-and -post sexual selection based on HLA.

It is difficult to produce a distinct conclusion based on the papers on recurrent spontaneous abortions or pregnancy loss. The reviewed papers showed evidence for the theory that homozygosity of certain HLA alleles, or other alleles in linkage disequilibrium enhances the chance of RSA, namely the HLA-DRB1*03, HLA-G*0106 and HLA-C2 alleles. Yet papers within distinct populations have opposing results, and other non HLA immune genes play an important role as well. Investigation into the precise effect of HLA-genes on success of pregnancy are frequently performed, and should be reviewed in full to come to a consensus.
Albeit all the literature discussed was not fully in agreement, a conclusion can still be made. Overall, the HLA region appears influential on pair bonding and sexual selection mostly via odour preference and cryptic female mate choice. This thesis shows the importance of olfaction on attraction and shows how genetics subtly affect mate selection and relationship satisfaction in humans.

Sources

1. Allen, C, Havlíček, J, Williams, K, Roberts, SC. (2018). Perfume experts' perceptions of body odors: Toward a new lexicon for body odor description. J Sens Stud.; 33:e12314.
2. Allen, C., Havlíček, J., Williams, K.S., Roberts, C. (2019). Evidence for odour-mediated assortative mating in humans: The impact of hormonal contraception and artificial fragrances, Physiology & Behavior,Volume 210, https://doi.org/10.1016/j.physbeh.2019.05.002 .
3. Arora, J., Pierini, F., McLaren, P. J., Carrington, M., Fellay, J., Lenz, T. L. (2020). HLA heterozygote advantage against HIV-1 is driven by quantitative and qualitative differences in HLA allele-specific peptide presentation. Molecular biology and evolution, 37(3), 639-650.
4. Berlingerio, M., Bonchi, F.,& Curcio, M., Giannotti, F., Turini, F. (2009). Mining Clinical, Immunological, and Genetic Data of Solid Organ Transplantation. 10.1007/978-3-642-02193-0_9.
5. Beydoun, H. Saftlas, A. (2005), Association of human leucocyte antigen sharing with recurrent spontaneous abortions. Tissue Antigens, 65: 123-135.
6. Chaix, R., Chen Cao, Donnelly, P. (2008). Is Mate Choice in Humans MHC-Dependent? PLoS Genetics, 4(9), 1–5., https://doi-org.proxy-ub.rug.nl/10.1371/journal.pgen.1000184
7. Elbaşı, M. O., Tulunay, A., Karagözoğlu, H., Kahraman, S., Ekşioğlu-Demiralp, E. (2020). Maternal killer-cell immunoglobulin-like receptors and paternal human leukocyte antigen ligands in recurrent pregnancy loss cases in Turkey. Clinical and experimental reproductive medicine, 47(2), 122–129.
8. Ejsmond, J.M., Radwan, J., Wilson, A.B. (2014). Sexual selection and the evolutionary dynamics of the major histocompatibility complexProc. R. Soc. B.2812014166220141662
9. Ferdenzi C, Richard Ortegón S, Delplanque S, Baldovini N, Bensafi M. (2020). Interdisciplinary challenges for elucidating human olfactory attractiveness. Phil. Trans. R. Soc. B 375, 20190268
10. Firman, R. C., Gasparini, C., Manier, M. K., Pizzari, T. (2017). Postmating Female Control: 20 Years of Cryptic Female Choice. Trends in ecology & evolution, 32(5), 368–382.
11. Fitzpatrick, J. L., Willis, C., Devigili, A., Young, A., Carroll, M., Hunter, H. R., Brison, D. R. (2020). Chemical signals from eggs facilitate cryptic female choice in humans. Proceedings of the Royal Society B, 287, 20200805
12. Flegel, C., Vogel, F., Hofreuter, A., Schreiner, B.S., Osthold, S., Veitinger, S. (2016). Characterization of the olfactory receptors expressed in human spermatozoa. Front Mol Biosci 2:73
13. Freeman-Gallant, C. R., Meguerdichian, M., Wheelwright, N. T., Sollecito, S.V. (2003). Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird. Molecular ecology, 12(11), 3077-3083.
14. Garver-Apgar, C. E., Gangestad, S. W., Thornhill, R., Miller, R. D., Olp, J. J. (2006). Major Histocompatibility Complex Alleles, Sexual Responsivity, and Unfaithfulness in Romantic Couples. Psychological Science, 17(10), 830–835.
15. Hakkarainen, T. J., Krams, I., Coetzee, V., Skrinda, I., Kecko, S., Krama, T., Ilonen, J., Rantala, M. J. (2021). MHC Class II Heterozygosity Associated With Attractiveness of Men and Women. Evolutionary psychology : an international journal of evolutionary approaches to psychology and behavior, 19(1), 1474704921991994.
16. Hämmerli, A., Schweisgut, C. Kaegi, M. (2012), Population genetic segmentation of MHC-correlated perfume preferences. International Journal of Cosmetic Science, 34: 161-168.

17. Havlíček, J., Roberts, S. C. (2009). MHC-correlated mate choice in humans: a review. Psychoneuroendocrinology, 34(4), 497–512.
18. Hillgarth, N. (1990). Parasites and Female Choice in the Ring-necked Pheasant, American Zoologist, Volume 30, Issue 2, Pages 227–233
19. Hviid, T. V., Hylenius, S., Hoegh, A. M., Kruse, C., Christiansen, O. B. (2002). HLA-G polymorphisms in couples with recurrent spontaneous abortions. Tissue antigens, 60(2), 122–132.
20. Jacob, S., McClintock, M., Zelano, B., Ober, C. (2002). Paternally inherited HLA alleles are associated with women's choice of male odor. Nat Genet 30, 175–179
21. Jokiniemi, A., Magris, M., Ritari, J., Kuusipalo, L., Lundgren, T., Partanen, J., Kekäläinen J. (2020). Post-copulatory genetic matchmaking: hla-dependent effects of cervical mucus on human sperm function. Proceedings. Biological Sciences, 287(1933), 20201682–20201682.
22. Klein, J., Sato, A. (2000b). The HLA system. First of two parts. The New England Journal of Medicine, 343(10), 702–709. https://doi-org.proxy-ub.rug.nl/10.1056/nejm200009073431006
23. Kromer, J., Hummel, T., Pietrowski, D., Giani, A.S., Sauter, J., Ehninger, G., Schmidt, A.H., Croy, I. (2016). Influence of HLA on human partnership and sexual satisfaction, Scientific Reports, 6, art. no. 32550.
24. Kreyden, O.P., Böni, R., Burg, G. (2002). Hyperhidrosis and Botulinum Toxin in Dermatology.Curr Probl Dermatol. Basel, Karger, vol 30, pp I-XII (DOI:10.1159/000060694)
25. Kruse, C., Steffensen, R., Varming, K., & Christiansen, O. B. (2004). A study of HLA-DR and-DQ alleles in 588 patients and 562 controls confirms that HLA-DRB1* 03 is associated with recurrent miscarriage. Human reproduction, 19(5), 1215-1221.
26. Kuukasjarvi, S., Eriksson, C.J.P., Koskela, E., Mappes, T., Nissinen, K., Rantala, M.J. (2004). Attractiveness of women's body odors over the menstrual cycle: the role of oral contraceptives and receiver sex. Behav. Ecol. 15, 579-584. (doi:10.1093/beheco/arh050)
27. Leinders-Zufall, T., Brennan, P., Widmayer, P., Chandramani, P., Maul-Pavicic, A., Jager, M., Li, X.-H., Breer, H., Zufall, F., Boehm, T. (2004). MHC class I peptides as chemosensory signals in the vomeronasal organ. Science, 306(5698), 1033–1037.
28. Lewis, M. B. (2010). Why are Mixed-Race People Perceived as More Attractive? Perception, 39(1), 136–138. https://doi.org/10.1068/p6626
29. Lübke, K., Schablitzky, S., Pause, B.M. (2009). Male sexual orientation affects sensitivity to androstenone. Chemosens Percept 2:154–160
30. Maciej, J.E., Jacek, T., Wilson, A. B. (2014). Sexual selection and the evolutionary dynamics of the major histocompatibility complexProc. R. Soc. B.2812014166220141662
31. Magris, M., Jokiniemi, A., Kuusipalo, L., Ritari, J., Koskela, S., Partanen, J., Kekäläinen J. (2021). Structural dissimilarity of partners' immune genes increases sperm viability in women's reproductive tract. Journal of Evolutionary Biology, 2021 May 31.
32. Mcclelland, E., Penn, D., Potts, W. (2003). Major Histocompatibility Complex Heterozygote Superiority during Coinfection. Infection and immunity. 71. 2079-86. 10.1128/IAI.71.4.2079-2086
33. McGann, J. P. (2017). Poor human olfaction is a 19th-century myth. Science (New York, N.Y.), 356(6338), eaam7263.
34. Meuleman, T., Lashley, L. E., Dekkers, O. M., van Lith, J. M., Claas, F. H., Bloemenkamp, K. W. (2015). HLA associations and HLA sharing in recurrent miscarriage: A systematic review and meta-analysis. Human Immunology, 76, 362–373. https://doi.org/10.1016/j.humimm.2015.02.004
35. Milinski, M. (2003). The function of mate choice in sticklebacks: optimizing MHC genetics. Journal of Fish Biology, 63, 1-16.
36. Milinski, M., Croy, I., Hummel, T., Boehm, T. (2013). Major histocompatibility complex peptide ligands as olfactory cues in human body odour assessment. Proc R Soc B 280: 20122889.

37. Milinski, M., Wedekind, C. (2001). Evidence for MHC-correlated perfume preferences in humans. Behavioral Ecology, 12(2), 140-149.
38. Natsch. A., Emter. R. (2020). The specific biochemistry of human axilla odour formation viewed in an evolutionary context. Philos. Trans. R. Soc. Lond. Ser. B 375:20190269 10.2210/PDB6SLF/PDB
39. Nowak, I., Malinowski, A., Tchórzewski, H., Barcz, E., Wilczyński, J.R., Banasik, M., Gryboś, M., Kurpisz, M., Łuszczek, W., Majorczyk, E., Wiśniewski, A., Senitzer, D., Sun, J.Y., Kuśnierczyk, P. (2011). HLA-C C1C2 heterozygosity may protect women bearing the killer immunoglobulin-like receptor AA genotype from spontaneous abortion, Journal of Reproductive Immunology, Volume 88, Issue 1, Pages 32-37,
40. Ober, C., Weitkamp, L.R., Cox, N., Dytch, H., Kostyu, D., Elias, S. (1997). HLA and Mate Choice in Humans, The American Journal of Human Genetics, Volume 61, Issue 3, Pages 497-504, https://doi.org/10.1086/515511.
41. Parham, P. Ohta, T. (1996). Population biology of antigen presentation by MHC class I molecules. Science 272, 67–74
42. Penn, D.J., Potts, W.K. (1999). The evolution of mating preferences and major histocompatibility complex genes, Am. Nat., 153, pp. 145-164
43. Qiao, Z., Powell, J. E., Evans, D. M. (2018). Mhc-dependent mate selection within 872 spousal pairs of european ancestry from the health and retirement study. Genes, 9(1). https://doi.org/10.3390/genes9010053
44. Roberts, S.C., Gosling, L.M., Carter, V., Petrie, M. (2008). MHC-correlated odour preferences in humans and the use of oral contraceptives. Proc Biol Sci. Dec 7;275(1652):2715-22. doi: 10.1098/rspb.2008.0825. PMID: 18700206; PMCID: PMC2605820.
45. Roberts, S.C., Little, A.C., Gosling, L.M., Jones, B.C., Perrett, D. I., Carter, V., Petrie M. (2005). MHC-assortative facial preferences in humansBiol. Lett.1400–403
46. Santos P.S.C., Schinemann, J.A., Gabardo, J., Bicalho, M.D. (2005). New evidence that the MHC influences odor perception in humans: a study with 58 Southern Brazilian students. Horm. Behav.;47:384–388.
47. Saphire-Bernstein, S., Larson, C. M., Gildersleeve, K. A., Fales, M. R., Pillsworth, E. G., Haselton, M. G. (2017). Genetic compatibility in long-term intimate relationships: partner similarity at major histocompatibility complex (MHC) genes may reduce in-pair attraction. Evolution and Human Behavior, 38(2), 190-196.
48. Sarolidou, G., Axelsson, J., Kimball, B.A., Sundelin, T., Regenbogen, C., Lundström, J.N., Lekander, M., Olsson, M.J. (2020). People expressing olfactory and visual cues of disease are less liked. Phil. Trans. R. Soc. B 375, 20190272. (doi:10.1098/rstb.2019.027)
49. Sereshki, N., Andalib, A., Ghahiri, A., Mehrabian, F., Sherkat, R., Rezaei, A., Wilkinson, D. (2019). The expression of human leukocyte antigen by human ejaculated spermatozoa. Molecular Genetics & Genomic Medicine, 7, 1–8.
50. Singh, P. B., Herbert, J., Roser, B., Arnott, L., Tucker, D. K., Brown, R. E. (1990). Rearing rats in a germ-free environment eliminates their odors of individuality. Journal of chemical ecology, 16(5), 1667–1682.
51. Stoyanov, G. S., Matev, B. K., Valchanov, P., Sapundzhiev, N., Young, J. R. (2018). The Human Vomeronasal (Jacobson's) Organ: A Short Review of Current Conceptions, With an English Translation of Potiquet's Original Text. Cureus, 10(5), e2643.
52. Tizard, I., Skow, L. (2021). The olfactory system: The remote-sensing arm of the immune system. Animal Health Research Reviews, 1-12.
53. Wedekind, C., Füri, S. (1997). Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity?. Proceedings of the Royal Society of London. Series B: Biological Sciences, 264(1387), 1471-1479.

54. Wedekind, C., Seebeck, T., Bettens, F., Paepke, A. (1995). MHC-Dependent Mate Preferences in Humans. Proceedings: Biological Sciences, 260(1359), 245-249. Retrieved June 11 2021
55. White, T.L., Cunningham, C. (2017). Sexual Preference and the Self-Reported Role of Olfaction in Mate Selection. Chem. Percept. 10, 31–41
56. Winternitz, J., Abbate, J.L., Huchard, E., Havlíček, J. and Garamszegi, L.Z. (2017), Patterns of MHC-dependent mate selection in humans and nonhuman primates: a meta-analysis. Mol Ecol, 26: 668-688.
57. Wobst, B., Zavazava, N., Luszyk, D., Lange, K., Ussat, S., Eggert, F., Ferstl, R., Müller-Ruchholtz, W. (1998). Molecular forms of soluble HLA in body fluids: potential determinants of body odor cues. Genetica, 104(3), 275–283.
58. Xu, X., Zhou, Y., Wei, H. (2020). Roles of HLA-G in the Maternal-Fetal Immune Microenvironment. Frontiers in immunology, 11
59. Xue, S., Yang, J., Yao, F., Xu, L. and Fan, L. (2007), Recurrent spontaneous abortions patients have more −14 bp/+14 bp heterozygotes in the 3′UT region of the HLA-G gene in a Chinese Han population. Tissue Antigens, 69: 153-155.
60. Yamazaki, K., Boyse, E. A., Mike, V., Thaler, H. T., Mathieson, B. J., Abbott, J., Thomas, L. (1976). Control of mating preferences in mice by genes in the major histocompatibility complex. The Journal of experimental medicine, 144(5), 1324-1335.
61. Younger, R. M., Amadou, C., Bethel, G., Ehlers, A., Lindahl, K. F., Forbes, S., Beck, S. (2001). Characterization of clustered MHC-linked olfactory receptor genes in humans and mice. Genome research, 11(4), 519-530.