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Major histocompatibility complex-linked social signalling affects female fertility

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Running head: Effects of MHC sharing on fertility

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Abstract
Genes of the major histocompatibility complex (MHC) have been shown to influence social signalling and mate preferences in many species, including humans. First observations suggest that MHC signalling may also affect female fertility. To test this hypothesis, we exposed 191 female horses (*Equus caballus*) to either a MHC-similar or a MHC-dissimilar stimulus male around the time of ovulation and conception. A within-subject experimental design controlled for non-MHC linked male characteristics, and instrumental insemination with semen of other males (N=106) controlled for potential confounding effects of semen or embryo characteristics. We found that females were more likely to become pregnant if exposed to a MHC-dissimilar than to a MHC-similar male, while overall genetic distance to the stimulus males (based on microsatellite markers on 20 chromosomes) had no effect. Our results demonstrate that early pregnancy failures can be due to maternal life-history decisions (cryptic female choice) influenced by MHC-linked social signalling.

Introduction
The major histocompatibility complex (MHC) is a group of polymorphic genes that play a crucial role in the adaptive immune response of vertebrates [1]. The MHC also plays an important role in social signalling, from parent-progeny and other kinds of kin recognition to mate choice and further contexts of inter-sexual communication [2, 1]. Ruff et al. [2] list over 20 species (including humans, see also [3, 4]) where some form of MHC social signalling could be identified, and further examples have been added since their review, including the horse (*Equus caballus*) [5, 6]. In the case of

MHC-dependent mate choice, the typical observation is that mating with MHC similar individuals is avoided and some degree of MHC-dissimilarity is preferred [2, 1] (see refs [7, 8] for a recent discussion of alternative models). MHC-linked social signalling is either based on either volatile chemical signals [e.g. 9] or on non-volatile MHC peptide ligands [10, 11]. The signals are recognized in the vomeronasal organ [10] and/or in the main olfactory system [12, 3], i.e. there seem to exist several independent mechanisms of MHC-linked social signalling, but the full pathways have not been solved yet.

In the context of sexual selection, the MHC may either be used as a marker for kinship to avoid inbreeding, or MHC-based mate preferences may serve to enhance the frequency of heterozygotes or of rare alleles among offspring [13] (even if MHC heterozygotes may not do better against a given infection than the respective homozygotes [14], they often show superiority during coinfections [15, 16]).

Mate choice is only one of several possible levels at which sexual selection may influence offspring genotype [2]. Cryptic female choice includes selection against certain types of sperm within the female reproductive tract [17, 18], non-random gamete fusion [19, 20], and non-random second meiotic division in the egg after gamete fusion [19, 21]. The possibility that MHC-linked signals affect female decisions at the earliest stages of a pregnancy, i.e. before implantation in the endometrium, has received little attention despite its potential relevance in mammals [22]. The frequency of early pregnancy failure can be high, e.g. around 22% in humans when diagnosed on daily urine samples, compared to 9% that happened after clinical detection of pregnancy [23]. If some of these early pregnancy failures were indeed due to maternal life-history decisions influenced by MHC-linked social signalling, human couples sharing MHC alleles would be expected to have longer periods of unprotected intercourse until a pregnancy would be diagnosed. This prediction was confirmed in studies on Hutterites [24], a group of people whose doctrine generally prohibits contraception. Ober *et al.*'s [24] observations suggest that cryptic female rejection of early embryos could be a form of sexual selection that contributes to the excess of MHC heterozygotes that is often observed in, for example, human populations [25]. Here we test this possibility, using horses (*Equus caballus*) as experimental model, and concentrating on European warmbloods to avoid potential breed effects.

Methods
Experimental procedures
The experimental infrastructure consisted of a corridor and 8 boxes (Fig. 1a). Over a period of five years, in total 191 oestrous mares without foal (only Warmblood, including 18 Franches-Montagnes horses, i.e. a Warmblood-related local breed) were individually stabled in one of the boxes during 62 hours (after feeding and water installations had been cleaned with water, faeces and urinated litter in the boxes had been removed, and new straw had been added). The mares were exposed to one of 10 stimulus stallions (all Franches-Montagnes) who could freely move in his box and the corridor for 17 hours/day. These stimulus stallions were all non-related and unfamiliar to the mares, and the mares were haphazardly assigned to them. There were usually several mares in the same stable (up to seven of the eight boxes were occupied with mares).

Before a mare would be exposed to a stimulus stallion, her cycle activity was monitored daily by rectal ultrasonographic examinations. When at least one follicle reached a diameter of ≥ 35 mm, a pronounced uterus oedema was present, and the

absence of any corpus luteum was confirmed, 1500 IU human chorionic gonadotropin (hCG; Chorulon®, Intervet, Boxmeer, Netherlands) were applied intravenously the following evening (in order to induce an ovulation approximately 36 - 42 h later [26]). The mare was then introduced into the experiment, i.e. exposed to a stimulus stallion. Instrumental inseminations were performed 24h and 38h after hCG application with fresh or frozen-thawed semen (of one of 106 other breeding stallions). Instrumental insemination allowed controlling for potential effects of semen characteristics [5] and of embryo genetics. Sixty-two hours after hCG injection, i.e. before blastocysts are expected to leave the oviduct [27], mares were stabled elsewhere without contact to any stallions. They were examined for pregnancy 14 - 17 days after ovulation by transrectal ultrasound [28].

MHC and microsatellite typing
Equine leukocyte antigens (ELA) were determined serologically as in Burger et al. [5, 6] in microcytotoxicity tests with alloantisera detecting 18 internationally recognised MHC class I ELA-A alleles (A1 – A10, A14, A15, A19, W11, W16, W17, W18, and W20), 5 locally defined specificities belonging to this same locus (Be22, Be25, Be27, Be28, Be108), the non-ELA-A class I antigens BeIII and W21, the MHC class II alleles DW13, DW22, DW23, DBe200 and DBeVIII, and the antigen W12 that could not yet be assigned to class I or II. Briefly, peripheral blood lymphocytes were obtained by Ficoll density gradient centrifugation, washed twice in PBS, resuspended in RPMI, and diluted to 2×10^6 cells/ml. Two µl of cell suspension were added to wells on Terasaki typing plates. After 20 min incubation at room temperature, 2 µl rabbit complement was added to each well. Visualisation of the reaction was performed 1 h later by adding 5 µl eosin followed by 5 µl formaldehyde for fixation. A positive reaction led to killing of more than 50% of target cells. ELA was determined in all 191 mares, all 10 stimulus stallions, and 31 of the 106 semen donors. MHC sharing to the semen donor could be determined for 80 mares. All MHC types were determined after the experiments, i.e. they were not known during handling of the animals. Most mares shared either 0 or 1 antigens with the stimulus stallion, but sharing of up to 3 antigens could be found. Of the 112 mares that shared at least one antigen with the stimulus stallion, 4 shared only one class II antigen, 14 shared class II and class I antigens, and the rest only class I antigens, i.e. MHC sharing refers mostly to sharing of class I antigens here. Franches-Montagnes (FM) mares were not more likely than other Warmblood (WB) to share MHC antigens with the FM stimulus stallions (there was even a non-significant tendency of lower sharing within FM than between WB and FM; FM mares: 39% sharing, WB mares: 61% sharing, $\chi^2 = 3.1$, p > 0.05).

Genetic similarity between individuals was estimated from 20 polymorphic microsatellite loci located on 20 different chromosomes (Supplementary Table S1). Genomic DNA was extracted from blood (EDTA) with the Qiagen BioSprint robotic workstation. Markers were amplified with GoTaq® DNA polymerase except AHT36, UMNe567 and UD457 which were pooled and amplified with QIAGEN® Multiplex PCR Kit. PCR reactions with GoTaq® DNA polymerase were performed in 10 µl reaction volume using 1X GoTaq green reaction buffer, 0.5 µl of DNA, 2.5 mM $MgCl_2$ in total, a mix of 0.2 mM dNTPs, 0.5 µM of each primer, and 0.25u of GoTaq® DNA polymerase. PCR program with varying annealing temperatures and a general procedure was used as follows: 4 min of initial denaturation at 94°C, 38 cycles of 30 s at 94°C, 60 s at optimal annealing temperature, 40 s at 72°C, then 5 min of final extension at 72°C. PCR with QIAGEN® Multiplex PCR Kit was performed in 10 µl

reaction volume using 1x QIAGEN Multiplex PCR Master Mix (3mM Mg^{2+}), 1 µl of DNA, 0.2 µM of each primer. PCR program was used as follows: 15 min of initial denaturation and activation step at 94°C, 38 cycles of 30 s at 94°C, 90 s at 60°C, 30 s at 72°C, then 10 min of final extension at 72°C. All amplicons were subsequently analysed on an ABI-3100 sequencer and allele sizes scored using the size standards ROX-350 (Genemapper 4.0, Applied Biosystems).

Typing of microsatellite loci was based on new blood samples taken in some cases long after the experiments. Some samples could no more be taken because the mare had meanwhile died or because of other constraints. Therefore, the microsatellite-related analyses were based on a haphazardly reduced sample of mares. Pairwise relatedness was calculated after Wang [29] using the R package Demerelate [30] based on all 20 microsatellite loci (we had the full genotypes of all stimulus stallions and of 126 mares).

We constructed generalized linear models (GLM) and generalized linear mixed models (GLMM) on pregnancy (yes/no) as dependent variable, with MHC sharing and genetic relatedness based on microsatellite diversity as fixed factors, and the identity of the stimulus stallion as random factor. In order to test the significance of an effect, a model lacking or including an effect was compared to a reference model in likelihood ratio tests. The analyses were performed in R 3.3.3 [31] with the lme4 package [32], and in Jmp® 11.2 (www.jmp.com).

Results

Sharing of at least one MHC antigen between mares and stimulus stallion was frequent (58.6%) and significantly reduced the rate of pregnancies after instrumental insemination (Table 1a, Fig. 1b). The identity of the stimulus stallion did not seem to play role here (Table 1a), and excluding the 18 mares of the Franches-Montagnes breed (a Warmblood-related local breed that was included in this study) did not change the conclusion that MHC sharing affected pregnancy (Supplementary Table S2). As expected, MHC sharing between mares and semen donors did not correlate to MHC sharing between mares and stimulus stallions (n= 80 mares; $\chi^2 = 0.7$, p = 0.40). Whether fresh or frozen-thawed semen was used did not significantly affect pregnancy rate (likelihood ratio test: $\chi^2 = 1.3$, p = 0.26) nor did mare age (logistic fit: $\chi^2 = 0.9$, p = 0.35).

There was no linkage disequilibrium among the 20 microsatellite markers (Figure S1; as expected from microsatellites that are located on different chromosomes). Table S3 provides the observed and the expected heterozygosity per locus. In total 8 of the 20 loci showed an excess of homozygotes (average $F_{is} = 0.078$; Table S3) that was partly due to a genetic differentiation between Warmblood and Franches-Montagnes horses (mean $F_{st} = 0.078$; Table S3). Average pairwise relatedness r between stimulus stallion and mare was -0.111 (± 0.137 s.d.). This indicator of relatedness was no significant predictor of MHC sharing (mean r of MHC-dissimilar pairs = -0.09 ± 0.019 (s.e.), of MHC-similar pairs = -0.12 ± 0.016; t = -1.2, p = 0.22) and did not predict pregnancy after the instrumental fertilization (Table 1b, Fig. 1c). When directly comparing the effects of the MHC and genetic distance on microsatellites, we found again MHC sharing to be a significant predictor of female fertility, while r showed no effects (Table 1c).

Figure 2 suggests that the number of shared MHC antigens did not play a role on the outcome of the instrumental fertilization. Nevertheless, models that compare the effects of MHC and r based on numbers of shared MHC antigens instead of the

dichotomous sharing/no sharing still confirm that MHC signalling affects pregnancy (Tables S4 and S5).

Discussion

Feral mares live in, or disperse between, social groups ("bands") that usually include one or two stallions, i.e. male-male dominance is important in this species but female choice by dispersal is possible and likely [33, 34]. Moreover, horses have long gestation periods (approximately 11 months), and foal condition crucially depends on maternal investment for some time after gestation [35]. Differential maternal reproductive strategies are therefore expected [36] and have indeed been found at early stages of pregnancy [37, 38]. Our findings demonstrate that differential maternal reproductive strategies can also depend on MHC-linked signals emitted by stallions. There may be other male traits that signal, for example, health and vigour or male-male dominance and that could potentially influence female reproductive decisions [39, 40]. Our experimental design separated these other potential effects from effects of MHC sharing. It turned out that no stimulus stallion was significantly superior in affecting pregnancy rates. What mattered was whether stimulus stallions and mares shared MHC antigens.

In mice, post-mating exposure to MHC-linked odours from a male who is not the progenitor can induce pregnancy termination [41]. This so-called "Bruce effect" [42-44] serves females to avoid costs of embryogenesis if offspring are likely to be killed by a new territory holder [45]. Evidence for the Bruce effect has been reported in horses [46, 38] and other equids [47], and it is possible that such pregnancy terminations are induced via MHC signalling in social communication, too. However, the selection mechanism we discovered here is different. A pregnancy termination in anticipation of male infanticide would require a stimulus that implies a take-over of a territory or band by a new male, i.e. a female would have to compare new male stimuli to the ones she received at the time of conception. If MHC-linked signals were involved here, the stimulus male's MHC would have to be different to the MHC of the genetic father of the embryo. Whether the stimulus male is similar or dissimilar to the female's MHC would not be expected to matter in such situations, as experimentally confirmed in mice [48]. However, the very early pregnancy failures that we observed here happened before embryonic implantation and pregnancy can be diagnosed by ultrasonography [49]. They were directly dependent on whether the mare shared MHC antigens with the stimulus stallion, i.e. they mirrored mate choice decisions that have been found in various vertebrates [2].

In humans, the prevalence of recurrent spontaneous abortions (of clinically diagnosed pregnancies) could frequently be linked to higher than usual MHC sharing between couples, but the evidence remains mixed [50, 51]. The kind of selection we found here would mostly go unnoticed or only delay menses in humans [23]. Maternal life-history decisions could play a role here, because human couples sharing MHC alleles have been found to have longer periods of unprotected intercourse between diagnosed pregnancies in Hutterites [24]. These first observations in humans [24] and our experimental findings in horses suggest that cryptic female acceptance or rejection of early embryos is an important further stage at which the MHC influences sexual selection.

In conclusion, cryptic female choice allows females to control male reproductive success after mating. In mammals, cryptic female choice may even include maternal decisions about survival of early embryos in the endometrium. If so, mate preferences are expected to influence female fertility. We tested this hypothesis

with horses, controlling for potentially confounding effects of ejaculate characteristics, embryo genetics, and genetic distance between mare and stimulus stallion. We found that mares are more likely to become pregnant if exposed to MHC-dissimilar than MHC-similar stallions around the time of instrumental insemination. It remains to be shown whether these negative effects of MHC-similar stallions on female reproductive decisions have evolved as a means to avoid inbreeding or to promote heterozygosity in the MHC region [13].

Ethics. Ethical clearance was granted by the Vaud canton, Switzerland (*Service Vétérinaire*, permission 2539 and 2227). Daily experimental handling time of mares and stallions was minimized. No manipulation resulted in injuries.

Data accessibility. The datasets supporting this article can be accessed at Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.04k5q

Supplementary Information. Tables S1 – S5; Figure S1.

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Table 1. Effects of genetic similarity on female fertility (pregnant yes/no).
Likelihood ratio tests comparing GLMMs and GLMs with genetic markers (fixed factors) and/or stallion identity ("ID", random factor) and reference models (indicated in italics) to test the effects of **(A)** MHC antigen sharing ("MHC", yes/no), and **(B)** pairwise genetic relatedness "r" based on 20 polymorphic microsatellite loci. **(C)** Direct comparison of effects of MHC and r in the subsample of 126 mares that allow for such a test. Significant p-values are emphasized in bold.

Model	Effect tested	d.f.	logL	χ^2	P
(A) MHC effects (191 mares)					
MHC + ID		3	-126.4		
ID	MHC sharing	2	-129.2	5.47	**0.019**
MHC	Stallion ID	2	-126.5	0.14	0.71
MHC + ID + IDxMHC	Stallion ID x MHC	4	-126.4	0.12	0.73
(B) Diversity on microsatellites (126 mares)					
r + ID		3	-85.5		
ID	r	2	-85.7	0.54	0.46
r	Stallion ID	2	-85.5	0	1.0
r + ID + ID x r	Stallion ID x r	4	-85.5	0	1.0
(C) MHC vs microsatellites (126 mares)					
MHC + r + ID		4	-79.9		
r + ID	MHC sharing	3	-85.5	11.2	**<0.001**
MHC + ID	r	3	-80.0	0.1	0.70
MHC + r	Stallion ID	3	-79.9	0	1.0

Figure Legends

Fig. 1. Experimental set-up and female fertility in response to the sharing of MHC or microsatellite alleles. (A) Exposure of oestrous mares (in boxes) to one of the 10 stimulus stallions. **(B)** Pregnancy rate per stimulus stallion (means ± 95% CI) in response to MHC sharing between mares (N=191) and stimulus stallions. **(C)** Logistic plot illustrating that pairwise relatedness r is a poor predictor of pregnancy. To improve visibility, points are jittered randomly along the y axis within the range that corresponds to pregnant or not pregnant. See Table 1 for statistics.
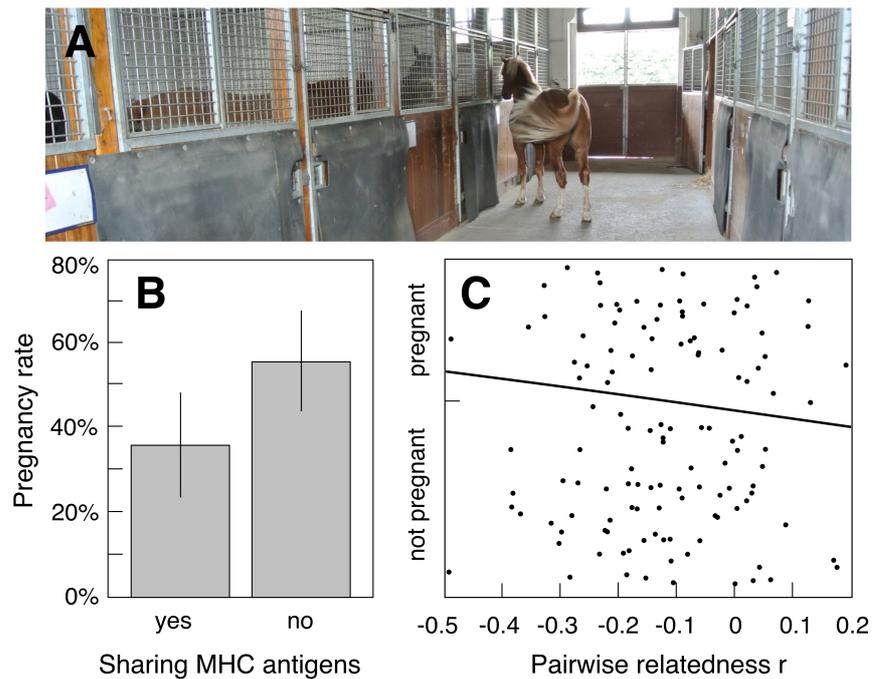
Fig. 2. Pregnancy rates relative to number of MHC antigens shared between stimulus stallion and mare. The numbers in the boxes give the number of mares.
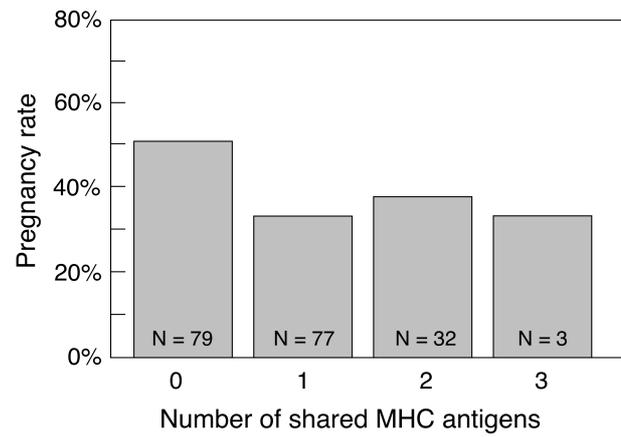
Supplementary Table S1. The 20 microsatellite markers used to determine overall genetic similarity
The name of the markers, their chromosomal location, their primer sequences [52], the annealing temperature, and the fluorophores we used, the number of alleles found in the present sample (N = 148), and the number of alleles previously observed in 311 world-wide distributed Hanoverian warmblood horses.

Name (chromosome)	Primer sequence (5'- 3')	Annealing temperature	Fluorophore	Number of alleles in present study	Number of alleles in Mittmann *et al*. [52]
VIAS-H34 (1)	GCTTTTGTTTCTCAATCCTAGC TGAAGTCAAATCCCTGCTTC	58°C	HEX	11	7
UMNe448 (2)	CCATTCTGCCCTGATTGG TTCAAGACCCCTCAATCTGC	55°C	FAM	6	6
AHT36 (3)	TGCTGCTCCAGTGTCCT TAGATTTCACAGGCGGGTG	60°C	AT550	9	8
UMNe567 (5)	GGTGCAGCTGCTAGCTCAG AGACCCAGTCATTGGGAGG	60°C	FAM	6	4
LEX065 (6)	GAAGGCACAATTCAATCTACT GCCCAGTCCCATTCTAAC	60°C	ATTO550	6	5

HTG4 (9)	CTATCTCAGTCTTCATTGCAGGAC CTCCCTCCCTCCCTCTGTTCTC	55°C	AT550	6	6
COR048 (10)	GATTGGGATGCAAAGATGAG CAAGAGGATTGGGAACAAAGG	58°C	FAM	8	6
UCD457 (11)	GGGGCGTGAGCATAAAGG CGCTGGATGAGTGAGGGA	60°C	FAM	12	10
COR069 (13)	AGGCAGCTTGACTACCCTGA AAAGTCTCCCCTGCGTGTT	58°C	ATTO550	8	7
UM010 (14)	TACAGCCATTGGAAATCTAC CACCATTACATTTTCCCAG	55°C	FAM	7	7
HMS20 (16)	TGAGTGTTTGCGTGTGTGTG TCCCGTCTCCTCTCTTGTTC	58°C	FAM	6	5
TKY924 (17)	TTCACCTATGAGTTTGAGGTA CGTCATAATGCAGACTCTTTG	55°C	HEX	5	4
TKY101 (18)	TCTGAAATACCGTGTGCCT TTCTGCCTCCCTCCAACTTT	55°C	FAM	9	8

TKY582 (22)	AGCCACCAGTCTGTTCTCTG	58°C	HEX	6	7
	AATGTCCTTTGGTGGATGAAC				
UCD405 (25)	ACCTCGTCTGGCTGTTGTAAG	60°C	HEX	9	9
	ACTTGCTGTGCGACTCTG				
UM005 (26)	CCCTACCTGAAATGAGAATTG	55°C	HEX	7	7
	GGCAAAAGATCAGGCCAT				
TKY315 (27)	GATGCCTCGAACTAGCTTG	55°C	FAM	8	8
	GATCTTCCATGTTTTTGTTTGG				
TKY333 (28)	CCTTCACTAGCCTTCAAATG	55°C	FAM	11	8
	TTGTGTTTAGACAGTGCTGC				
COR082 (29)	TGGGAGAGGTACCTGAAATGTAC	55°C	ATTO550	9	7
	GTTGCTATAAAAAATTGTCTCCCTAC				
AHT34 (31)	CTCAGGGCGAATGTTCCTC	60°C	FAM	9	7
	CCCCACCATGAGTCAAAAC				

Supplementary Table S2. Effects of genetic similarity on female fertility (pregnant yes/no), restricting the analyses to only warmblood mares (N=173). Likelihood ratio tests comparing GLMMs and GLMs with genetic markers (fixed factors) and/or stallion identity ("ID", random factor) and reference models (indicated in italics) to test the effects of **(A)** MHC antigen sharing ("MHC", yes/no), and **(B)** pairwise genetic relatedness "r" based on 20 polymorphic microsatellite loci. **(C)** Direct comparison of effects of MHC and r in the subsample of 113 mares that allow for such a test. Significant p-values are emphasized in bold.

Model	Effect tested	d.f.	logL	χ^2	P
(A) MHC effects (173 mares)					
MHC + ID		3	-113.8		
ID	MHC sharing	2	-115.5	3.3	0.07[1]
MHC	Stallion ID	2	-113.9	0.02	0.90
MHC + ID + ID x MHC	Stallion ID x MHC	4	-133.7	0.3	0.58
(B) Diversity on microsatellites (113 mares)					
r + ID		3	-75.0		
ID	r	2	-75.1	0.24	0.63
r	Stallion ID	2	-75.0	0	1.0
r + ID + ID x r	Stallion ID x r	4	-75.0	0	1.0
(C) MHC vs microsatellites (113 mares)					
MHC + r+ ID		4	-70.4		
r + ID	MHC sharing	3	-75.0	9.1	**0.003**
MHC + ID	r	3	-70.7	0.6	0.44
MHC + r	Stallion ID	3	-70.4	0	1

[1] p = 0.04 if tested directed (Rice and Gaines 1994 *PNAS.* **91,** 225-226) based on the *a priori* expectancy that MHC-dissimilar stallions are more attractive than MHC-similar stallions

Supplementary Table S3. Diversity of the 20 microsatellite loci.

Observed (H_{obs}) and expected (H_{exp}) heterozygosity, test for deviation from Hardy-Weinberg equilibrium (χ^2, d.f., p), heterozygosity deficiency coefficient (F_{is}), and differentiation between Warmblood and Franches-Montagnes horses (F_{st}).

Name	H_{obs}	H_{exp}	χ^2	d.f.	p	F_{is}	F_{st}
AHT36	0.749	0.770	179.7	45	0*	0.047	0.044
UCD457	0.682	0.740	220.2	78	0.014	0.097	0.074
UMNe567	0.538	0.657	49.1	15	0*	0.181	0.009
HTG4	0.610	0.651	158.3	21	0.135	-0.027	0.099
TKY101	0.528	0.696	200.4	45	0*	0.212	0.001
TKY315	0.585	0.641	26.1	28	0.107	0.104	0.051
COR082	0.728	0.769	181.3	45	0.086	0.008	0.071
UCD405	0.677	0.739	57.5	36	0.084	0.088	0.055
UMO10	0.790	0.755	22.3	21	0.291	-0.076	0.009
COR069	0.703	0.743	84.1	28	0*	0.030	0.086
HMS20	0.631	0.679	163.5	15	0.002*	-0.016	0.263
LEX065	0.713	0.675	7.4	15	0.947	-0.051	0.008
TKY582	0.641	0.772	172.2	21	0*	0.127	0.229
AHT34	0.790	0.804	63.1	36	0.049	0.007	0.116
UMNe448	0.672	0.743	24.5	15	0.015	0.155	0.023
COR048	0.641	0.690	18.4	28	0.218	0.098	0.115
TKY333	0.779	0.830	72.9	55	0.289	0.035	0.055
UM005	0.697	0.772	177.6	21	0.002*	0.098	0.120
TKY924	0.764	0.731	6.4	10	0.693	-0.060	0.054
VIAS.H34	0.379	0.732	688.1	55	0*	0.499	0.027

*significant after Holm-Bonferroni correction

Supplementary Table S4. Effects of genetic similarity on female fertility (pregnant yes/no) based on numbers of shared MHC antigens instead of the dichotomous sharing/no sharing. Likelihood ratio tests comparing GLMMs and GLMs with genetic markers (fixed factors) and/or stallion identity ("ID", random factor) and reference models (indicated in italics) to test the effects of **(A)** number of shared MHC antigens ("MHC", range 0 to 3), and **(B)** direct comparison of effects of number of shared MHC antigens and pairwise relatedness r in the subsample of 126 mares that allow for such a test. Significant p-values are emphasized in bold.

Model	Effect tested	d.f.	logL	χ^2	P
(A) MHC effects (191 mares)					
MHC + ID		3	-127.6		
ID	MHC sharing	2	-129.2	3.2	0.07
MHC	Stallion ID	2	-127.6	0.1	0.80
MHC + ID + IDxMHC	Stallion ID x MHC	4	-127.6	0.1	0.81
(B) MHC vs microsatellites (126 mares)					
MHC + r + ID		4	-80.0		
r + ID	MHC sharing	3	-85.5	10.9	**0.001**
MHC + ID	r	3	-80.1	0.03	0.86
MHC + r	Stallion ID	3	-80.0	0	1.0

Supplementary Table S5. Effects of genetic similarity on female fertility (pregnant yes/no), based on numbers of shared MHC antigens instead of the dichotomous sharing/no sharing and restricting the analyses to only warmblood mares (N=173). Likelihood ratio tests comparing GLMMs and GLMs with genetic markers (fixed factors) and/or stallion identity ("ID", random factor) and reference models (indicated in italics) to test the effects of **(A)** number of shared MHC antigens ("MHC", range 0 to 3), and **(B)** the effects of the number of shared MHC antigens and r in the subsample of 113 mares that allow for a direct comparison. Significant p-values are emphasized in bold.

Model	Effect tested	d.f.	logL	χ^2	P
(A) MHC effects (173 mares)					
MHC + ID		3	-114.7		
ID	MHC sharing	2	-115.5	1.6	0.20
MHC	Stallion ID	2	-114.7	0	1.0
MHC + ID + ID x MHC	Stallion ID x MHC	4	-114.6	0.1	0.78
(B) MHC vs microsatellites (113 mares)					
MHC + r + ID		4	-70.6		
r + ID	MHC sharing	3	-75.0	8.8	**0.003**
MHC + ID	r	3	-71.0	0.9	0.35
MHC + r	Stallion ID	3	-70.6	0	1

Supplementary Figure S1. Testing for linkage disequilibrium between the 20 microsatellite loci. There was no linkage disequilibrium among the 20 microsatellite markers.