Archives of Andrology, 52:45–50, 2006

ISSN: 0148-5016 print/1521-0375 online
DOI: 10.1080/01485010500316519

EVIDENCE FAVORING SPERM SELECTION OVER SPERM COMPETITION IN THE INTERACTION BETWEEN HUMAN SEMINAL PLASMA AND SPERM MOTILITY *IN VITRO*

K. Jaffe, M. I. Camejo, T. E. Carrillo, M. Weffer, and M. G. Muñoz □ *Departamento de Biología de Organismos, Universidad Simón Bolívar, Caracas, Venezuela*

□ *The effect on sperm motility of sperm-sperm and sperm-seminal plasma interactions was studied among homologous and heterologous sperm. There were no significant interactions between sperm in vitro, but it was found that seminal plasmas of different donors have different effects on sperm motility, and different sperm react differently to the same seminal plasma. Sperm showed higher motility in a pure physiological solution than when mixed with seminal plasma, even if the plasma and sperm came from the same donor. Various plasma components are responsible for this modulation of sperm motility. It would appear that large numbers of sperm are adaptive, among other things, because they are involved in sperm selection.*

Keywords sperm, competition, selection, human

INTRODUCTION

Males of most animal species produce large numbers of sperm [4]. What is the adaptive value of these huge numbers? We do not know for certain yet, and several theories have tackled the subject directly or indirectly [5, 14, 17]. Yet two theories explain this feature: the sperm competition and the sperm selection theory. The sperm competition theory [1, 2, 17, 18, 23] assumes that large numbers of sperm may have evolved as a result of competition between males for fertilizing the ova. More sperm will increase the likelihood of a successful fertilization. Mechanisms, such as large testis, sperm plugs, or killer sperm, might be expected if sperm competition is at work. In contrast, sperm selection [4] is defined as a means to weed out unfit male haplotypes before

We thank G. Arata de Bellabarba, D. Ajami, E. Herrera, C. Bosque, O. Arenas, R. Apitz, M.T. Bosque and several referees for helpful comments.
Address correspondence to K. Jaffe, Departamento de Biología de Organismos, Universidad Simón Bolívar, Apartado 89000, Caracas 1080, Venezuela. E-mail: kjaffe@usb.ve

fertilization [12, 13]. This selection acts upon the phenotype expressed in sperm through spermiogenesis [15, 21] before chromatin condensation. Mechanisms promoting sperm selection will evolve naturally in sexual organisms because they accelerate natural selection by selecting good haplotypes without the need for an organism to develop to maturity [13]. Sperm selection also provides strong selection pressure for sex to stabilize as an adaptive mechanism during evolution [10, 11]. Biochemical and physiological mechanisms that eliminate unfit sperm will increase the odds of success for the offspring, accelerating evolution by strengthening natural selection at a low cost to the parent. Another proposed mechanism of gamete selection is genetic compatibility [25]. In both proposals, seminal plasma (and vaginal fluids) provide an environment where sperm with a low deleterious mutation load and with a good working biochemical-metabolic engine that depends on critical proteins [16] are more likely to survive. Selection of sperm is equivalent, in evolutionary terms, to mate selection [12], but is more economic in time and energy expenditure, enhancing the beneficial aspects of sex [13].

As a proxy for sperm fertility we used sperm motility exists as evidence for a correlation between fertility and sperm motility. The logic of the experiments was based on the interactions that might be expected if sperm competition and/or sperm selection have modulated human reproductive physiology. These are summarized as follows:

	Expected effect on sperm motility for the following hypotheses:			
Interaction	Sperm competition	Sperm selection	Competition +selection	No competition no selection
Heterologous: sperm-sperm	Reduction	No effect	Reduction	No effect
Homologous: plasma-sperm	No effect	Reduction	Reduction	No effect
Heterologous: plasma-sperm	Reduction	Reduction	Reduction	No effect

MATERIALS AND METHODS

Semen Sample

Semen samples were collected by masturbation of 36, 18–24 year students. The samples were processed within one hour of collection. Sperm motility was measured in each sample using a semi-automatic system [5]. A drawing tube was introduced between the objective and the ocular of the microscope to track the movement of sperm manually with the help of a cursor on a digitizing board, and the data was processed with a computer [8]. For each sample, 50 randomly selected sperm were analyzed

[26]. As linear sperm motility correlates with fertility, only the percentage of sperm showing linear motility is processed here.

Interactions Between Swim-up Sperm of Two Different Men

Progressively motile sperm were isolated after they migrate out of seminal plasma into the overlying layer of equal volume of BWW medium [3], supplemented with 0.3% bovine serum albumin, incubating it at 37°C for 60 min and then collecting the migrate sperm. The concentrations were adjusted to 10^6 sperm ml^{-1}. Combinations of 18 pairs of different sperm samples were used for each set of experiments. Each pair of donors took their samples at the same day and within a time span of a maximum 1 hr. A mix of 50 μl of each sample was incubated in a tube for 1 hr at 37°C. Then the sperm motility was measured in each sample. This result was compared with the motility of swim-up sperm of the same man (homologous interaction), with that of the mix of two sperm donors (heterologous interaction), and with the calculated average of the two values for sperm motility when sperm of each donor was tested singly (the expected heterologous result).

Interactions Between Swim-up Sperm and Heterologous Seminal Plasma

Seminal plasmas of 17 healthy men, obtained after semen centrifugation, were stored in individual aliquots at 20°C. Sperm from 3 healthy donors (D1, D2, D3) were obtained by the swim-up technique. Sperm of each of the 3 men were then incubated with each of 17 seminal plasmas (heterologous interactions) for 3 min at 37°C. Another fraction of each sperm sample was placed into a pool of all 17 seminal plasmas tested. The percentage of sperm showing linear motility was compared with the motility of the sperm in the seminal plasma of its donor male (homologous interaction).

Interactions Between Swim-up Sperm and Seminal Plasma Fractions of High (H) and Low (L) Molecular Weight

Fractions of seminal plasma of three donors were obtained by ultrafiltration at high pressure with a Diaflo membrane permeable to molecules $<$10.000 MW and were then re-suspended in BWW medium. The donors of these seminal plasmas (SP-3, SP-7 and SP-14) were chosen from a previous experiment for their extreme values of sperm motility in heterologous sperm-plasma interactions compared to homologous sperm-plasma interaction.

The experiment consisted of testing 5 μl of sperm obtained by swim-up from three healthy donors (D1, D2, D3), by immersing them in 5 μl of full seminal plasma, or in the high molecular weight fractions of the seminal plasma, or low molecular weight fraction from the donors. Sperm were incubated in the plasma samples for three minutes at 37°C. The percentage of sperm showing lineal motility was evaluated. The results were compared with the motility of sperm placed in their own full seminal plasma (homologous sperm-plasma interaction)

RESULTS

There was no effect on sperm motility when sperm from one donor where mixed with that from another donor without the presence of seminal plasmas. The percentage of motile swim-up sperm in the mix (heterologous sample) was compared with that expected by calculating the average of the sperm motility of each of the two swim-up samples assessed when isolated (homologous sample) by subtracting the latter from the former. This difference is compared with the expected outcome. A difference of 0% is expected if no interaction between heterologous sperm occurs. There was no significant difference evident between sperm motility in these two conditions ($T = 16$, $p = 0.139$, $n = 18$). Thus, sperm motility of a given donor is similar if measured in isolation or mixed with sperm from another donor, but only if no seminal plasma is present [19].

When the motility of sperm from a given donor was assessed in a preparation containing seminal plasmas from two donors, statistically significant effects on sperm motility due to the mix could be detected. That is, the percentage of motile sperm was significantly lower (Wilcoxon's matched pairs test: $T = 4$, $p = 0.002$, $n = 18$) compared to the expected motility under homologous conditions (sperm in seminal plasma of its donor) if no interaction between heterologous sperm occurs. The expected motility in this case was the average of the two values for sperm motility obtained when each sperm sample was placed in the plasma from the donor that produced them. Thus, the presence of sperm plasma significantly altered the results of sperm mixes when counting percentages of motile sperm.

When swim-up sperm of each donor were placed in a pool of seminal plasma from all 17 donors, sperm motility dropped markedly compared to the average sperm motility of the 17 sperm samples measured under homologous conditions ($T = 14.5$, $p = 0.017$). Thus, the presence of plasma inhibits sperm motility even in a homologous scenario. Different seminal plasma affected the sperm motility differently. In order to investigate these differences in more detail, 17 plasma samples were taken again, randomly choosing the donors from those providing the data just presented. The plasmas were screened for their ability to affect the sperm motility of three donors, selected because their plasmas showed the strongest reaction. For example, if we take the swim-up sperm of D1 and place them in homologous seminal plasma (SP-own) 58% of the sperm show linear movement.

Two of the seminal plasmas tested (SP-3 and SP-14) showed the most extreme responses on sperm of the donors. SP-3 reduced the motility, whereas SP-7 increased the motility of sperm from all three donors. Seminal plasma SP-14 had irregular effects on the three sperm samples tested. These three plasmas were chosen to fractionate them in order to study the effect on sperm motility of plasma fractions of different molecular weight. These plasmas were dialyzed, as described in the materials and methods section, and the fractions obtained were tested separately from the sperm from all three donors (D1, D2, D3), which are different individuals to those providing the seminal plasma (SP-3, SP-7 and SP-14). Thus, motility depended on the compound's in each fraction and not on the sperm.

DISCUSSION

Seminal plasma-sperm interactions occur even in homologous sperm ejaculates [20]. Variations in this interaction are not due to statistical noise or experimental artifacts, as the cause of the variance could be isolated either in low or high molecular weight fractions. These various factors in the seminal plasma interact with sperm in different ways. Experiments using mixes of two or of several seminal plasmas showed that the various putative factors affecting sperm motility do not cancel each other out; rather, increased numbers of seminal plasmas in the mix decrease the proportion of sperm moving adequately.

Sperm selection has been neglected as a relevant process because of several reasons. One is that phenotypic expression of haplotipes is poorly understood. Another reason is that it is often believed that sperm's phenotype are determined by the genotype of the diploid parent rather than their own haploid genomes, so that all the phenotypes of sperm from a single individual are identical. This assumes that the haploid genotype lies dormant and is not expressed. This mechanism has been proposed as a way to prevent selfish alleles competing within the ejaculate [9]. Although the working of such a mechanism cannot be excluded completely, it does not apply for all genes in the genome as spermiogenesis requires a minimum of haploid-genome expression [7, 21, 22, 24]. Even if only a few genes are expressed phenotypically, and these genes are central to basic protein networks [16], sperm selection would be an important driving force of evolution in sexual organisms [13]. Other physiological and developmental mechanisms, such as germline competition [6], would also achieve a similar result.

Most seminal plasma immobilize sperm from the same donor, i.e., that seminal plasma is toxic for most sperm. This finding cannot be explained by sperm competition theory, but such an outcome is expected if sperm selection mechanisms are at work, as the plasma should weed out defective sperm.

REFERENCES

1. Baker RR, Bellis MA (1995): Human sperm competition. London: Chapman & Hall.
2. Birkhead TT, Møller AP (Eds). (1998): Sperm competition and sexual selection. NewYork, NY: Academic Press, p 826.
3. Brinster RL (1965): Studies on the development of mouse embryos *in vitro*. IV. Interaction of energy sources. J Reprod Fert 10:227–240.
4. Cohen J (1999): Sperms galore: why mammals produce large numbers of spermatozoa. Science Spectra 16:14–20.
5. Comhaire F, Vermeulen L (1995): Human semen analysis. Hum Reprod Update 1:343–362.
6. Hastings IM (1989): Potential germline competition in animals and its evolutionary implications. Genetics 123:191–197.
7. Hecht NB (1995): The making of a spermatozoon: a molecular perspective. Dev Genetics 16:95–103.
8. Hinting A, Schoonjans F, Comhaire F (1988): Validation of a single step procedure for the objective assessment of sperm motility characteristics. Int J Androl 11:227–287.
9. Hurst LD, Peck JR (1996): Recent advances in the understanding of the evolution and maintenance of sex. Trend Ecol Evol 11:46–52.
10. Jaffe K (1999): On the adaptive value of some mate selection strategies. Acta Biotheoretica 47:29–40.
11. Jaffe K (2000): Emergence and maintenance of sex among diploid organisms aided by assortative mating. Acta Biotheoretica 48:137–147.
12. Jaffe K (2002): On sex, mate selection and evolution: an exploration. Comm Theor Biol 7:91–107.
13. Jaffe K (2004): Sex promotes gamete selection: A quantitative comparative study of features favoring the evolution of sex. Complexity 9:43–51.
14. Jennions MD, Petrie M (2000): Why do females mate multiply? A review of the genetic benefits. Biol Rev Camb Philos Soc 75:21–64.
15. Jensen-Seaman MI, Li WH (2003): Evolution of the hominoid semenogelin genes, the major proteins of ejaculated semen. J Mol Evol 57:261–270.
16. Jeong H, Mason SP, et al. (2001): Lethality and centrality in protein networks. Nature 411:41–42.
17. Keller L, Reeve HK (1995): Why do females mate with multiple males? The sexually selected sperm hypothesis. Adv Study Behav 24:291–315.
18. Moore H, Dvorakova K, et al. (2002): Exceptional sperm cooperation in the wood mouse. Nature 418:174–177.
19. Moore HD, Martin M, Birkhead TR (1999): No evidence for the killer sperm or other selective interactions between human spermatozoa in ejaculates of different males in vitro. Proc R Soc London Biol Sci 266:2343–2350.
20. Mortimer S, Mortimer D (1988): Effect of seminal plasma on capacitation and hyperactivation in human spermatoza. Human Reproduction 13:2139–2146.
21. Oko R, Clemont Y (1999): Spermiogenesis. In: Encyclopedia of Reproduction, Knobil E, Neil JD, (Eds). Academic Press, vol. 4: pp 602–609.
22. Olds-Clarke P, Johnson LR(1993): T-haplotypes in the mouse compromise sperm flagellar function. Developmental Biology 155:14–25.
23. Parker GA (1970): Sperm competition and its evolutionary consequences in the insects. Biol. Rev 45:525–567.
24. Ren D, Navarro B, et al. (2001): A sperm ion channel required for sperm motility and male fertility. Nature 413:603–609.
25. Tregenza T, Wedell N (2000): Genetic compatibility, mate choice and patterns of parentage: invited review. Mol Ecol 9:1013–1027.
26. Vera O, Muñoz MG, Jaffe K (1998): Wave parameters of the sperm flagellum as predictors of human spermatozoa motility. Andrologia 30:153–157.