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Evidence favoring sperm selection over sperm competition in the interaction between human seminal plasma and sperm motility *in vitro*

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Abstract: The effect on sperm motility of sperm-sperm and sperm-seminal plasma interactions was studied among homologous and heterologous sperm. There was no significant interactions between sperm *in vitro* but 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 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 spermatozoa motility. It would appear that large numbers of spermatozoa are adaptive, among other things, because they allow the working of spermatozoa selection.

Introduction

Males of most animal species produce large numbers of spermatozoa [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 most theories can be placed on a continuum between two theories seeking to explain this feature: the sperm competition and the sperm selection theory. That is, emphasis on the organisms or on the cell, as the most important vehicle for selection to act in molding spermatozoa numbers.

The sperm competition theory [1,2,17,18,23] assumes that large numbers of spermatozoa may have evolved as a result of competition between males for fertilizing the ova of females. More spermatozoa 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 prior to fertilization [12,13]. This selection acts upon the phenotype expressed in spermatozoa 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 spermatozoa 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 spermatozoa with a low deleterious mutation load and with a good working biochemical-metabolic engine that dependents on critical proteins [16], are more likely to survive. Selection of spermatozoa is equivalent, in evolutionary terms, to mate selection [12], but is more economical in time and energy expenditure, enhancing the beneficial aspects of sex [13]. Thus, processes selecting spermatozoa should be favored by evolution.

These theories are not necessarily contradictory, as they might complement each other. In the present study, we designed experiments to evaluate the existence of mechanisms that might have been evolved by adaptive forces favoring sperm competition and/or sperm selection. We tested for possible spermatozoa-spermatozoa and/or spermatozoa-seminal plasma interactions that might affect spermatozoa motility, and thus, eventually, spermatozoa survival. As a proxy for sperm fertility we used spermatozoa motility, as evidence for a correlation between fertility and spermatozoa motility exist. 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:

Interaction	**Expected effect on sperm motility for the following hypotheses:**			
	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. Semen quality was screened for the criteria of normal human semen. Spermatozoa 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 spermatozoa 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 spermatozoa were analyzed as described in [26]. As linear spermatozoa motility is known to correlate with fertility, only the percentage of spermatozoa showing linear motility is processed here. Data from other parameters support the findings presented here (not shown).

Interactions between "swim-up spermatozoa" of two different men: Progressively motile spermatozoa were isolated after they migrate out of seminal plasma into 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 spermatozoa. The concentrations were adjusted to 10^6 spermatozoa ml^{-1}. Combinations of 18 pairs of different spermatozoa 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 maximum one hour. A mix of 50 µl of each sample was incubated in a tube for one hour at 37°C. Then the sperm motility was measured in each sample as described above. This result was compared with the motility of swim-up spermatozoa 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 spermatozoa motility when sperm of each donor was tested singly (the expected heterologous result).

Interactions between "swim-up spermatozoa" and heterologous seminal plasma: Seminal plasmas of 17 healthy men were obtained after semen centrifugation and were stored in individual aliquots at –20 °C. Spermatozoa from three healthy donors (D) (D1, D2, D3) were obtained by the swim-up technique. Spermatozoa of each of the three men were then incubated with each of the seventeen seminal plasmas (heterologous interactions) for three minutes at 37°C. Another fraction of each spermatozoa sample was placed into a pool of all 17 seminal plasmas tested. The percentage of spermatozoa showing linear motility was recorded and compare with the motility of the spermatozoa in the seminal plasma of its donor male (homologous interaction).

Interaction between "swim-up spermatozoa" 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 previous experiment for their extreme values of spermatozoa motility in heterologous spermatozoa-plasma interactions compared to homologous spermatozoa-plasma interaction.

The experiment consisted in testing 5 μl of spermatozoa 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. Spermatozoa were incubated in the plasma samples for three minutes at 37°C. Then the percentage of spermatozoa showing lineal motility was evaluated. The results were compared with the motility of spermatozoa placed in their own full seminal plasma (homologous spermatozoa-plasma interaction)

Results

Our experiments could not detect any effect on spermatozoa motility when spermatozoa from one donor where mixed with that from another donor without the presence of seminal plasmas. That is, we compare the percentage of motile “swim-up” spermatozoa in the mix (heterologous sample) with that expected by calculating the average of the spermatozoa motility of each of the two “swim-up” samples assessed when isolated (homologous sample) by subtracting the later from the former. This difference is compared with the expected outcome. A difference of 0 % is expected if no interaction between heterologous sperm occurs. Our results showed that no statistically significant difference was evident between spermatozoa motility in these two conditions ($T = 16$, $p = 0.139$, $n = 18$). Thus, spermatozoa motility of a given donor is similar if measured in isolation or mixed with spermatozoa from another donor, but only if no seminal plasma is present.

When the motility of spermatozoa from a given donor was assessed in a preparation containing seminal plasmas from two donors, statistically significant effects on spermatozoa motility due to the mix could be detected. That is, the percentage of motile spermatozoa was significantly lower (Wilcoxon´s matched pairs test: $T = 4$, $p = 0.002$, $n = 18$) compared to the expected motility under homologous conditions (spermatozoa 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 spermatozoa motility obtained when each spermatozoa 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 spermatozoa.

When "swim-up" spermatozoa of each donor were placed in a pool of seminal plasma from all 17 donors, spermatozoa motility dropped markedly compared to the average spermatozoa motility of the 17 sperm samples measured under homologous conditions ($T = 14.5$, $p = 0.017$, $n = 36$). When comparing the motility of "swim-up" spermatozoa swimming in their own plasma to the motility of "swim-up" spermatozoa in BWW, a clear inhibitory effect on movement of the plasma is evidenced. Thus the presence of plasma inhibits spermatozoa motility even in a homologous scenario.

Our results showed large variances. Although in average, or measured as a median, the spermatozoa motility decreased when heterologous sperm plasmas were present; this did not happed to all spermatozoa samples. Different seminal plasma affected the spermatozoa motility differently. In order to investigate these differences in more detail, 17 plasma samples were taken again, choosing randomly the donors from those providing the data just presented. The plasmas were screened for their ability to affect the spermatozoa motility of three donors, selected because their plasmas showed the strongest reaction. For example, if we take the "swim-up" spermatozoa of donor 1 (D1) and place them in homologous seminal plasma (SP-own), we obtain that 58 % of the spermatozoa show linear movement under our laboratory conditions. This value serves as reference. The same “swim-up” spermatozoa (another sample of the same ejaculate of donor 1), when placed in the seminal plasma of subject 17 (SP-17) will show only a 21 % of spermatozoa motility. Yet if spermatozoa from D1 are placed in SP-6, much more spermatozoa will move, producing a value of 81 % motility. The relative values will be (0.36 -1)* 100 = -64 % and (1.39-1)*100 = 39 % respectively.

A two way ANOVA with fixed effects performed on the data showed that the variability in the percentage of spermatozoa showing linear speed was determined as much by the donor of the spermatozoa ($F = 6.97$, $p = 0.0001$, $n = 17$) as by that of the plasma ($F = 2.79$, $p = 0.001$, $n = 17$). The plasmas of the subjects had different effects on the spermatozoa of the three donors. For example, spermatozoa from D1 reduced their motility when tested in plasma SP-14, whereas those of D3 increased their motility when tested in the same plasma SP-14.

Two of the seminal plasmas tested (SP-3 and SP-14) showed the most extreme responses on spermatozoa of the donors. SP-3 reduced the motility, whereas SP-7 increased the motility, of spermatozoa from all three donors. Seminal plasma SP-14 had irregular effects on the three spermatozoa samples tested. These three plasmas were chosen to fractionate them in order to study the effect on spermatozoa motility of plasma fractions of different molecular weight. These plasmas were dialyzed, as described in materials and methods, and the fractions obtained were tested separately. The results show that the spermatozoa from all three donors (D1, D2, D3), which are different individuals to those providing the seminal plasma (SP-3, SP-7 and SP-14) react similarly in the presence of a fraction of a given sample, i.e. get activated or inhibited, although with different sensitivities. This result show that the motility measured seems to be more determined by the compounds in the plasma than by the properties of the spermatozoa. The various fractions had different effects on the motility of spermatozoa. The high molecular fraction of SP-7 and SP-14 activate the motility of the spermatozoa from all donors tested (D1, D2, D3) whereas that of SP-3 diminished it. All low molecular weight fractions diminished spermatozoa motility.

These last results serve as a control showing the robustness of our results. Sperm motility is affected by many factors, such as density, temperature, time of collection, duration of freezing and others which we could not control for 100 %. Despite this variability, the individuals for which we collected semen twice showed that in both cases they seminal plasma induced the same type of reaction on his spermatozoa and that from other donors. Thus, our results can not be explained away as methodological artifacts, but hint to an underlying phenomenon that needs to be explained.

Discussion

Although our experimental designs do not mirror exactly the physiological processes that might occur in humans, our results suggest that human spermatozoa do not interact with each other, and that the effect of homologous plasma on spermatozoa motility is not neutral. We might reject the hypothesis that human spermatozoa compete with each other, confirming previous studies [19]. Referring to the logical design of the study presented in the introduction, the evidence favors sperm selection theory against sperm competition, as forces that molded the reproductive physiology among humans.

We showed that seminal plasma interacts with spermatozoa. Seminal plasmas might activate or inhibit spermatozoa motility. This activation or inhibition might be due to the action of various different mechanisms which might express different results on sperm motility at different times. For example, excessive activation might immobilize spermatozoa after a while as they will exhaust their energy reserves, and the opposite might occur with inhibition. That seminal plasma affect spermatozoa motility had been reported many times in very different contexts [20 for just one example]. Here we show that seminal plasma-spermatozoa interactions occur even in homologous sperm ejaculates. 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 spermatozoa in different ways. Experiments using mixes of two or of several seminal plasmas showed that the various putative factors affecting spermatozoa motility do not cancel each other out; rather, increased numbers of seminal plasmas in the mix decrease the proportion of spermatozoa moving adequately.

Our results showed that most seminal plasma immobilize spermatozoa from the same donor, i.e that seminal plasma is toxic for most spermatozoa. This finding can not be explained by sperm competition theory; but such an outcome is expected if spermatozoa selection mechanisms are at work as the plasma should weed out defective spermatozoa. These facts and the relatively low occurrence of copula with multiple partners in short time periods among humans, suggests that spermatozoa selection may be much more important than sperm competition, at least among humans.

Spermatozoa 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 spermatozoa's phenotype are determined by the genotype of the diploid parent rather than their own haploid genomes, so that all the phenotypes of spermatozoa 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 can not be excluded completely, it clearly 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], spermatozoa 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.

Increased knowledge of the biochemical and immunological mechanisms that are selecting spermatozoa when semen plasma mixes with vaginal fluids (absent in *in vitro* fertilizations) could improve in vitro fertilization.

5. References

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