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Timing of sperm capacitation appears to be programmed according to egg availability in the female genital tract

The time course of the level of A23187-induced acrosome reaction between human and rabbit spermatozoa was compared. It was extended in the former (a periodic ovulator) and short in the latter (an induced ovulator). This finding suggests that the capacitated state is programmed to maximize the prospects that an ovulated egg will meet spermatozoa in the best functional state. (Fertil Steril® 2004;82:247–9. ©2004 by American Society for Reproductive Medicine.)

Key Words: Sperm capacitation, egg availability, capacitation, chemotaxis, acrosome reaction

To penetrate the egg and fertilize it, mammalian spermatozoa must be in a specific state of readiness known as capacitation. It was long believed that this state is rather static and prolonged, until it was demonstrated in human spermatozoa that only a small fraction of the sperm population is capacitated at any given time, that the capacitated state is transient (1–4 hours life span), that it occurs only once in the sperm's lifetime, and that different sperm individuals get to this stage at different time points, resulting in a continuous replacement of capacitated cells within the sperm population (1). For a review, see Jaiswal and Eisenbach (2). This finding was in line with earlier observations that only a small proportion of a sperm population is able to fertilize the egg in vivo (3), to undergo zona pellucida-stimulated acrosome reaction (4), to bind mannose (a suggested molecular marker of human sperm capacitation [5, 6]) under capacitating conditions (7, 8), or to be hyperactivated (9–12).

This information has created a dilemma because if the capacitated state is so important, why is its lifetime in humans so short and why are so few spermatozoa capacitated at any given time? We hypothesized that the role of these capacitation features is to prolong the time period during which capacitated spermatozoa can be found in the female genital tract, namely that the continuous replacement of capacitated cells is a mechanism, evolved in humans, to compensate for the lack of coordination between insemination and ovulation (1, 2, 13).

Because there is no direct way to examine this hypothesis, we employed an indirect approach. If our hypothesis is correct, the prolonged availability of capacitated spermatozoa should be advantageous for mammals that ovulate periodically like humans, where insemination is unlinked to ovulation. However, prolonged availability of capacitated spermatozoa would not be advantageous to mammals that ovulate in response to mating, like the rabbit. There, because the egg is always ovulated into a sperm-containing oviduct, a beneficial situation would be the availability of capacitated cells for a short period around ovulation. With this rationale in mind, and considering that only those spermatozoa that have completed the capacitation process are able to undergo an induced acrosome reaction (an exocytotic process that enables sperm penetration into the egg [2, 14]), we compared the time course of induced acrosome reaction between human and rabbit spermatozoa. Therefore, our criterion for the level of capacitated spermatozoa was the percentage of spermatozoa that underwent the acrosome reaction upon pharmacological stimulation (15). Here, we used the Ca^{2+} ionophore A23187, which is, according to the Andrology Special Interest Group of the European Society for Human Reproduction and Embryology (ESHRE), a commonly used acrosome-reaction inducer (16) that stimulates a complete acrosome reaction (15).

After removal of seminal plasma (human sperm by Percoll gradient and rabbit sperm by migration-sedimentation), the sperm concentration was adjusted to 1-2 $\times$ 10^6cells/mL in Biggers, Whitten and Whittingham medium (BWW) (17) with 4% of bovine serum albumin (BSA), and the cells were incubated at 37°C for the indicated periods. The percentage of A23187-induced, acrosome-reacted spermatozoa was determined from the difference between the levels of acrosome-reacted spermatozoa before and after an acrosome reaction induced by 30 minutes of incubation with the calcium ionophore, A23187 (10 μM). Acrosome-reacted spermatozoa were visualized by the acrosome marker Pisum sativum agglutinin labeled with fluorescein isothiocyanate, as previously described for humans (1) and rabbits (18). Assays were done according to the National Institutes of Health (NIH) guidelines for experimentation.

In humans, the proportion of A23187-induced, acrosome-reacted spermatozoa rose, without a delay, to a relatively low steady-state level that was retained throughout the measuring period of 30 hours, in line with an earlier study (1), whereas the percentage of motile cells hardly changed and remained high (Fig. 1A). This

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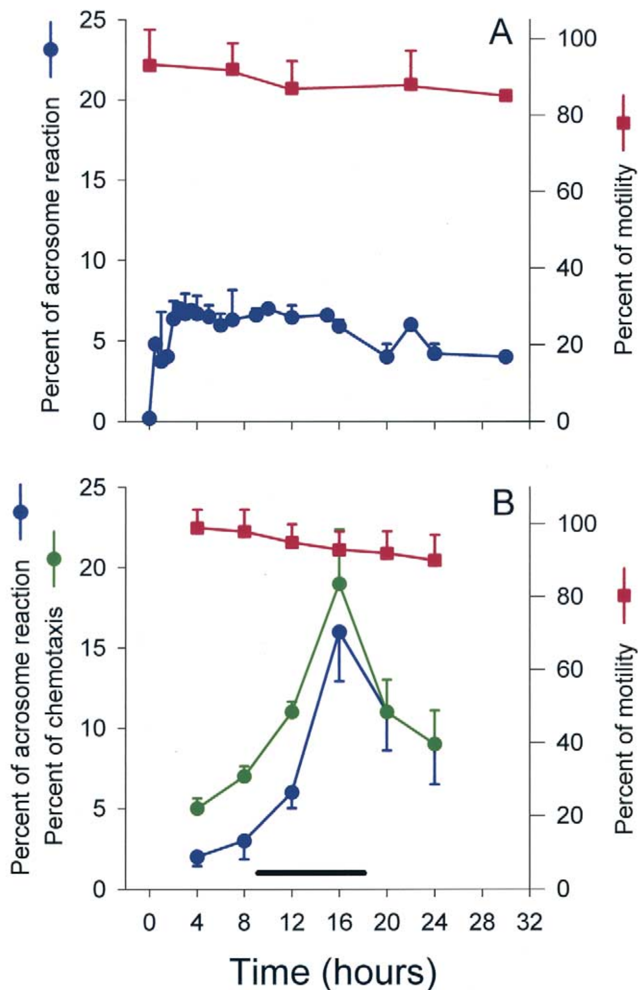
FIGURE 1

Comparison between the time-dependent levels of A23187-induced acrosome-reacted spermatozoa in humans (**A**) and rabbits (**B**). The results (mean ± SEM) are from 3 to 13 independent experiments, where 600 to 10,000 cells for capacitation and 200 cells for chemotaxis were analyzed in each time point of each experiment. Percentages were arcsen-square-root-transformed before the analysis of variance (ANOVA), then, the statistical differences were obtained by means of the *t*-test. The level of A23187-induced, acrosome-reacted human spermatozoa was significantly higher than the level at time zero after 2 hours of incubation ($P<.0001$). The rabbit's values at 12, 16, and 20 hours were significantly different from those at 4 and 8 hours ($P<.001$), and the points at 16 hours from those at 12 and 20 hours ($P<.001$). Horizontal bar: viability time of an ovulated egg in the rabbit oviduct (25).



Giojalas. Timing of sperm capacitation. Fertil Steril 2004.

rather long period of time at which acrosome reacted cells are available in a sperm population is in agreement with findings made with spermatozoa of other periodic ovulators such as dogs (19) and goats (20), and it fits the need to prolong the availability of fertilizing spermatozoa in humans and other periodic ovulators. Quantitative differences between various studies that measured the levels of acrosome reacted spermatozoa induced by different stimulants have been reported. Some examples can be found in Cross et al. (4), Cummins et al. (21), Krausz et al. (22), and Calvo et al. (23). However, those differences may be attributed to the different ways of semen processing, and the assays employed for the induction of the acrosome reaction, each likely measuring a different stage of capacitation and acrosome reaction (15).

In contrast, in the rabbit (an induced ovulator), the level of A23187-induced, acrosome-reacted spermatozoa was low for about 8 to 10 hours, after which it rose, reaching a maximum at 16 hours postejaculation, and then it declined (Fig. 1B). In another study, by employing P as an acrosome-reaction inducer instead of A23187, a maximal level of acrosome-reacted spermatozoa was similarly obtained at 16 hours (24). Because only capacitated spermatozoa can respond by chemotaxis to follicular fluid (1, 18), we used this property as an additional way to evaluate the level of capacitated cells. The time dependence of the level of chemotactically responsive cells was similar to that of the acrosome-reacted spermatozoa (Fig. 1B), as indicated by the high linear correlation coefficient ($r^2 = 0.90$; $n = 24$; $P<0.01$, according to the Pearson test). Here, too, the percentage of motile rabbit spermatozoa remained high (>90%) throughout the measurement period, indicating that the decrease in the level of induced acrosome-reacted and chemotactic cells after 16 hours was not due to cell death. This time window of the capacitated state in rabbits fits very well with the time window at which an egg can be found in the rabbit's oviduct, which is roughly 10 to 18 hours after ejaculation (25) (Fig. 1B, horizontal bar). It is also in line with early in vivo findings that spermatozoa recovered from the rabbit uterus 16 hours postcopulation have higher fertilizing potential than spermatozoa recovered after 12 or 24 hours (26, 27). Moreover, as might be expected, in the case of a rabbit female that undergoes multiple mating, the large majority of offspring belong to the first mating male — the one that induced the ovulation (28). Concerning other induced ovulators (e.g., cats and camels), no information is available.

Based on the shape of both A23187-induced acrosome-reaction curves plotted in Figure 1 (the absolute values may vary depending on the techniques and species), the observations made in this study support our hypothesis. The comparison between human and rabbit spermatozoa suggests that the timing and duration of the capacitated state is programmed in mammals according to the time of egg availability in the oviduct — extended in periodic ovulators and short in induced ovulators. This situation apparently maximizes the prospects that an ovulated egg would meet spermatozoa in the best functional state.

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