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Sperm Pairing in the Opossum Increases the Efficiency of Sperm Movement in a Viscous Environment[1]

H.D.M. MOORE[2,3] and D.A. TAGGART[4]

Department of Molecular Biology and Biotechnology,[3] University of Sheffield Sheffield S10 2UH United Kingdom
Department of Anatomy,[4] Monash University, Clayton 3168 Australia

ABSTRACT

In order to understand why sperm pairing has evolved in most American marsupials, the movement parameters of spermatozoa from *Monodelphis domestica* were analyzed after incubation in capacitating medium for 15 min, 2 h, and 24 h to induce a proportion of sperm pairs to uncouple. Motility characteristics of paired and single spermatozoa were measured in media of differing composition and viscosity by means of computer-aided semen analysis. In minimum essential medium or in RPMI 1640 medium alone, the absolute mean straight-line and curvilinear velocity values of paired spermatozoa (342 ± 34 and 361 ± 19 μm/sec, respectively, at 37°C) were significantly greater than those of single spermatozoa (247 ± 14 and 319 ± 16 μm/sec), while mean lateral head displacement for paired spermatozoa (5.6 ± 2.1 μm) was significantly less than for single spermatozoa (11.4 ± 2.6 μm). However, when medium was made more viscous with polyvinyl pyrrolidone (0.8–82 poise) and sperm motility was calculated as a percentage of maximum attained velocity (in medium alone), there was no significant difference in straight-line or curvilinear velocity for single or paired spermatozoa in medium of the lowest viscosity (0.8 poise). In contrast, paired spermatozoa in medium of higher viscosity (above 1.92 poise) maintained straight-line velocity (e.g., 54 ± 3% of maximum straight-line velocity in medium of 2.28 poise) while single sperm moved in tight circles and exhibited poor straight-line velocity (5 ± 1% of maximum velocity). The data show that paired spermatozoa exhibit a significant motility advantage over single spermatozoa in a viscous medium. A preliminary investigation of the fluid from the isthmic region of the oviduct of *Monodelphis* indicated that it was comparable with that of medium with a viscosity of < 13.5 poise. It is suggested that sperm pairing in marsupials evolved as a mechanism for efficient passage of spermatozoa along the viscous environment of the marsupial female genital tract.

INTRODUCTION

The morphology and function of the mammalian spermatozoon and the characteristics of the ejaculate are thought to have evolved as a result of underlying selective pressures determined in part by sperm competition and female selection [1]. For example, as a general rule, in species where sperm competition occurs, adult males have larger testes and produce more spermatozoa in the ejaculate than in equivalent species in which females mate with only one male. This occurs because, where multiple matings normally occur, the male that transfers the greater number of spermatozoa may be more likely to sire offspring [2]. Conversely, where the female selects a single male with which to mate and sperm competition is avoided (e.g. hopping mouse [3]), the total number of spermatozoa in the ejaculate may be relatively low, and sperm morphology is often pleiomorphic. In general, mammals ejaculate many more spermatozoa than seem to be needed for fertilization, and only a small proportion (e.g., rabbit, *ca* 0.01%) are found in the oviducts at the time of fertilization [4]. Ultimately, it is the relative success of competent spermatozoa to reach the site of fertilization that influences reproductive outcome rather than merely the total number inseminated. Efficient sperm passage through the female tract and across the egg vestments is determined by the relationship between spermatozoa and the tract environment. This is reflected in the morphology and motility characteristics of the spermatozoon in relation to the resistant forces of the female tract, i.e., viscosity of cervical and oviductal fluids, epithelial interactions, egg vestments [5].

American marsupials provide a unique opportunity to investigate aspects of sperm motility since, with the possible exception of one species (*Dromiciops australis* [6]), all form pairs of spermatozoa during epididymal maturation [7–9], which then function as a biflagellate unit [10]. Recent ultrastructural observations in the grey short-tailed opossum, *Monodelphis domestica* [11] indicate that, prior to final conjugation in the distal epididymis, spermatozoa undergo a complex rotation process enabling their heads to be exactly aligned such that about 80–90% of mature spermatozoa form pairs. In the occasional eutherian species, spermatozoa may align and stack to form a rouleaux of varying cell numbers (e.g., guinea-pig, [12]), but it is probably not coincidental that the only mammals to have evolved precise sperm pairing are marsupials. In these mammals, radical morphological reorganization of the sperm head accompanies maturation in the epididymis, thereby providing sufficient morphological plasticity for successful pairing to occur.

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[2]Correspondence: Professor H.D.M. Moore, Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2UH, UK. FAX: 0742 728697.

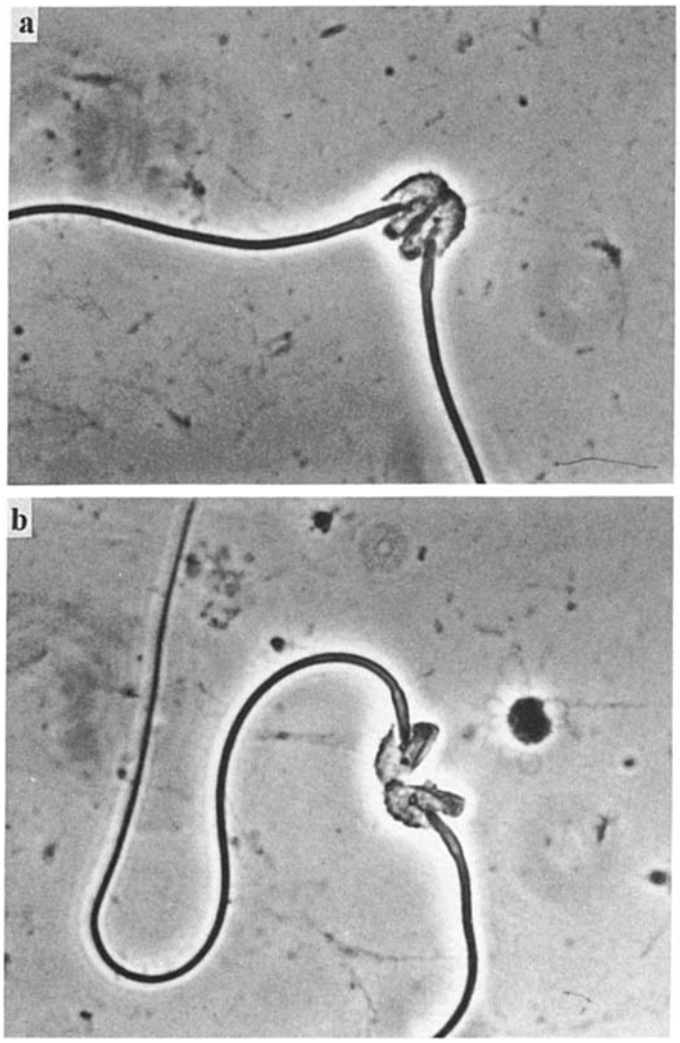

FIG. 1. Spermatozoa of *Monodelphis domestica*. a) Paired spermatozoa. Sperm heads are joined over their flat acrosomal surface to form a hydrodynamic biflagellate unit. b) Paired spermatozoa in process of separating. Spermatozoa remain motile after separation. ×580 phase contrast.

An intriguing question is: Do paired spermatozoa, compared with single spermatozoa, have an advantage in terms of motility and sperm transport in the female tract? Compared with most mammals, opossums have relatively small numbers of spermatozoa in the epididymis [4, 11] and consequently in their ejaculate, but a relatively high proportion (*ca* 5%) reach the oviduct prior to fertilization ([4]; Moore, unpublished observation). Sperm remain paired at ejaculation and during passage through the female tract to the lower isthmic region of the oviduct. After ovulation, paired spermatozoa pass into the ampulla region and then separate prior to fertilization [13, 14]. During in vitro incubation in capacitating medium, spermatozoa start to unpair after about 2 h and are then capable of binding to oocytes and undergoing the acrosome reaction [15]. In order to mimic the viscous environment of the female tract, we have examined the motility of paired and unpaired spermatozoa of the grey short-tailed opossum, *Monodelphis domestica*, in physiological media of differing viscosity.

TABLE 1. Composition and corresponding viscosity of medium used to assess sperm motility

Medium	Viscosity (poise)
Medium alone	0.69
Medium + 0.08% PVP	0.80
+ 0.15% PVP	0.92
+ 0.65% PVP	1.92
+ 1.0% PVP	2.28
+ 5.0% PVP	13.50
+ 10.0% PVP	81.70

MATERIALS AND METHODS

Animals and Maintenance

All the animals in this study were from a laboratory colony of grey short-tailed opossums originally established at the Institute of Zoology and currently held at the University of Sheffield. Housing, lighting and dietary requirements are described in detail elsewhere [16, 17]. Briefly, adult opossums were kept singly in rat cages (45 × 25 cm) at 25–28°C in 50–70% relative humidity and under artificial light (12L:12D with lights-on from 1900 to 0700 h). Diet consisted of a fox food pellet (Kemovit, Bristol, UK) given ad libitum and fresh minced beef given once a week.

Collection and Preparation of Caudal Epididymal Spermatozoa

Epididymal spermatozoa (Fig. 1) from mature males were collected as described previously and placed in RPMI 1640 (Life Technologies, Paisley, Scotland) or modified minimum essential medium (MEM) [15] with 25 mM HEPES, pH 7.4, in 400-μl drops under silicone oil (Dow Corning 200/200cs; BDH, Poole, UK) at 37°C or 30°C. Sperm concentration in the drops was 0.5–1.0 × 10^6 ml^{-1}.

Media of Differing Viscosity

To increase the viscosity of medium, polyvinyl pyrrolidone (PVP-10, Sigma Chemicals, (Poole, UK) was added to stock RPMI medium or MEM (see above) to give a range of viscosity as measured by an "Oswald" Viscometer (Scientific Instruments Ltd., London, UK). Viscosities were calculated by the formula:

η (viscosity) = specific $\text{gravity}_{(x)} \times t_{(x)} \times \eta_{(w)}/t_{(w)}$

where x = unknown, t = time in seconds, and w = water. The viscosities of the modified media at 37°C are shown in Table 1.

Assessment of Sperm Motility

Spermatozoa in drops of medium were replaced with test medium of a different viscosity. This could be done

TABLE 2. Straight line and curvilinear velocities, and lateral head displacements (± SD) of paired and single spermatozoa incubated in MEM and RPMI medium at 37°C and 30°C for 15 min.

	MEM medium				RPMI medium			
	30°C		37°C		30°C		37°C	
Sperm motility parameters	Paired*	Single	Paired*	Single	Paired*	Single	Paired*	Single
CASA measurements								
Straight-line vel (μm/sec)	292 ± 22	218 ± 18	342 ± 34	247 ± 14	288 ± 16	203 ± 17	367 ± 26	226 ± 25
Curvilinear vel (μm/sec)	324 ± 31	256 ± 23	361 ± 19	319 ± 16	333 ± 23	244 ± 19	389 ± 34	291 ± 14
Lateral head displ (μm)	6.2 ± 1.8	12.5 ± 4.1	5.6 ± 2.1	11.4 ± 2.6	6.4 ± 2.1	12.0 ± 3.6	5.4 ± 2.1	10.9 ± 5.4
Sperm track tracing								
Straight-line vel (μm/sec)	285 ± 68	222 ± 61	298 ± 48	230 ± 60	274 ± 71	219 ± 56	321 ± 57	224 ± 45
Curvilinear vel (μm/sec)	301 ± 72	243 ± 47	309 ± 48	282 ± 59	295 ± 68	205 ± 42	352 ± 71	285 ± 61
Lateral head displ (μm)	5.6 ± 3.1	11.9 ± 5.2	6.7 ± 3.8	10.4 ± 4.1	7.1 ± 3.5	13.6 ± 5.2	5.9 ± 2.7	10.5 ± 4.7

*Paired spermatozoa display significantly greater straight-line and curvilinear velocities, and lower lateral head displacement than single spermatozoa for each incubation ($p \leq 0.05$).

with minimal loss of spermatozoa because most cells attached by the head to the bottom of the culture plate. Spermatozoa were then resuspended in the drop of test medium by gentle pipetting, and an aliquot (50 μl) was transferred to prewarmed glass slides coated with 0.4% agar. The drops were covered with an agar-coated coverslip. To ensure that sperm motility was not restricted, glass beads measuring 100 μm in diameter were mixed in paraffin grease, and four beads were applied to the corners of the coverslip to support it and provide a sandwich of approximately 100 μm in depth. Motility was measured in the following preparations: 1) spermatozoa after 15-min incubation (> 80% sperm-pairs); 2) spermatozoa after 2-h incubation in modified MEM (< 60% sperm-pairs); 3) spermatozoa after 24-h incubation in modified MEM (< 20% sperm-pairs); and 4) spermatozoa recovered from viscous medium and transferred to stock medium (MEM only).

Two different methods of quantifying sperm motility were used. After preliminary experiments to establish the imaging thresholds, a computer-assisted sperm analyzer (CASA; Hobson Sperm Tracker, Sheffield, UK) was used to measure individual tracks of single or paired spermatozoa. The facility to display each track (trail-draw) was used to plot and record individual tracks. Sperm measurements were made directly from the drops of medium by means of an inverted microscope and video camera (Nikon, London, UK) at 120× magnification. In order to validate this system, a smaller number of observations (RPMI medium only) were made by undertaking video recordings of sperm motility and subsequently tracing sperm tracks (frame by frame) on acetate sheets placed directly on the monitor. Measurements were made of 25 spermatozoa in each RPMI medium. Distances traveled by spermatozoa were measured by use of a digitizer board (Apple, London, UK).

Statistical Analysis

Statistics were analyzed by means of the CSS Statistica program (Statsoft, London, UK) using ANOVA [18].

RESULTS

Sperm Motility in RPMI medium or MEM Without Viscosity Increase

By means of the CASA (Hobson Sperm Tracker System), 400–600 sperm tracks were analyzed for each time point and viscosity. Since individual sperm/sperm pairs were tracked, the number of tracks corresponded closely to the number of different sperm analyzed. Only a very limited number (25) of sperm tracks were determined by the tracing method for each incubation period. The curvilinear velocity, straight-line velocity, and lateral head displacement (mean values ± standard deviations) in medium at 37°C and 30°C (MEM only) for paired and unpaired spermatozoa incubated for 15 min are shown in Table 2. Paired spermatozoa displayed significantly greater ($p \leq 0.05$) veloci-

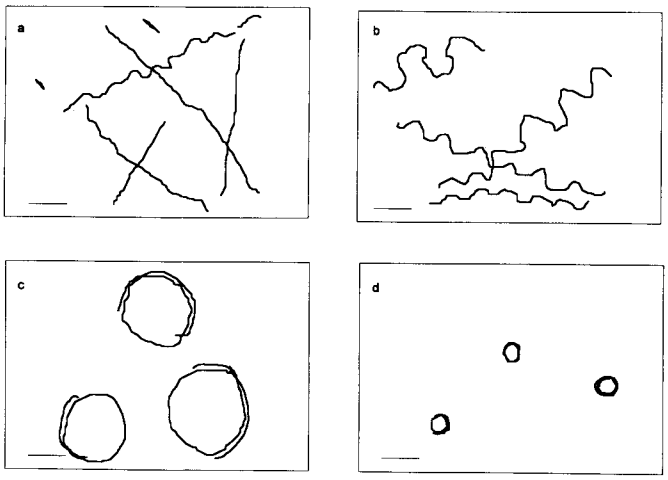

FIG. 2. Individual trails of paired or single spermatozoa after 2-h incubation as captured by "trail-draw" facility of Hobson Sperm Tracker. a) Paired sperm trails in MEM alone over 3 sec. b) Single sperm trails in MEM alone over 5 sec. c) Single sperm trails in MEM + 1% PVP over 7 sec. d) Single sperm trails in MEM + 5% PVP over 7 sec. Bar = 100 μm.

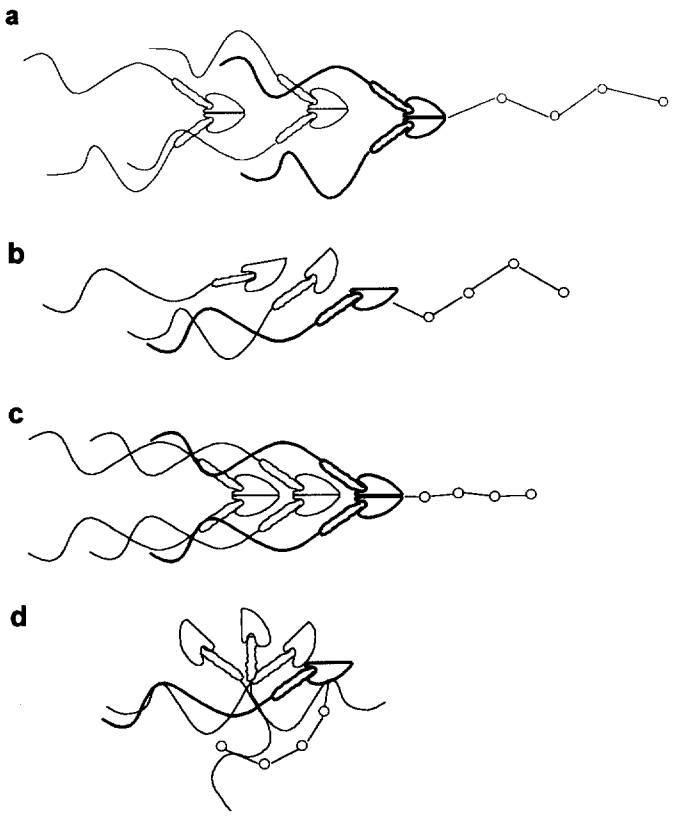

FIG. 3. Diagram depicting sperm movement in nonviscous (0.69 poise) and viscous (13.5 poise) medium. a) Paired spermatozoa in nonviscous medium. b) Single spermatozoon in nonviscous medium. c) Paired spermatozoa in viscous medium. d) Single spermatozoon in viscous medium

ties and lower lateral head displacement than did unpaired spermatozoa for each incubation period. However, there was no significant difference in motility parameters for spermatozoa in RPMI medium or MEM. Examination of sperm movement revealed that the paired spermatozoa moved with highly coordinated flagella beats with little rotational movement. In contrast, unpaired spermatozoa exhibited greater lateral head displacement and occasional rotational movement (see Figs. 2 and 3). Results of analysis of sperm tracks by tracing were not significantly different from those by the CASA although the standard deviation was large because only 25 measurements were made (Table 2).

Sperm Motility in Viscous Medium

The straight-line and curvilinear velocities of paired spermatozoa were significantly greater than those of single spermatozoa in each medium (Tables 2 and 3). Since spermatozoa in RPMI medium and MEM gave similar results, only the values for MEM are presented. To make comparisons between paired and single spermatozoa, their motilities in viscous medium were expressed as a proportion of their maximum velocity (straight-line or curvilinear) in medium without PVP. Calculated this way, the decline in the velocity of single spermatozoa with increasing viscosity was much greater than that of paired spermatozoa except at the viscosity of 0.8 poise, where there was no significant difference in the decline in sperm velocity when measured as a proportion of the maximum velocity. In medium containing 5% PVP (13.5 poise), single spermatozoa displayed little or no progressive motility (0–0.2%), whereas paired spermatozoa still maintained a straight-line velocity of between 19 and 37% of their maximum velocity in medium alone. The decrease in sperm velocity with increasing viscosity was temporary during the period of analysis with the CASA since replacing the PVP medium with medium alone restored the velocities of both paired and single spermatozoa to close to their original values.

Spermatozoa incubated for 2 h in capacitating medium prior to motility analysis displayed the greatest velocities compared with measurements after 15 min or 24 h incubation (Table 3). The differences in the straight-line velocity of paired and single spermatozoa were most pronounced at this time. At a viscosity of 2.28 poise (1% PVP), paired spermatozoa displayed a straight-line velocity of 56% of their maximum (Table 3). By contrast, single spermatozoa at the same viscosity moved at only 3% of their maximum velocity. The changes in curvilinear velocity with viscosity (Table 3) were not as dramatic as the straight-line velocities: spermatozoa continued to exhibit some movement in viscous media although this was not progressive motility. The typical patterns of sperm motility for paired

TABLE 3. Straight-line and curvilinear velocities of paired and single spermatozoa (as % of maximum velocity in medium alone) after 15 min, 2-h and 24-h incubation at 37°C.

	Velocity after 15 min (% maximum)				Velocity after 2 h (% maximum)				Velocity after 24 h (% maximum)			
Viscosity	Straight-line		Curvi-linear		Straight-line		Curvi-linear		Straight-line		Curvi-linear	
(poise)	Paired	Single	Paired	Single	Paired	Single	Paired	Single	Paired	Single	Paired	Single
0.80	95 ± 5	96 ± 6	97 ± 5	95 ± 7	94 ± 5	94 ± 6	94 ± 5	97 ± 7	97 ± 7	82 ± 5	93 ± 5	91 ± 5
0.92	89 ± 4	67 ± 6	89 ± 6	92 ± 5	90 ± 5*	56 ± 4	86 ± 5*	66 ± 4	76 ± 5*	20 ± 3	72 ± 4*	33 ± 2
1.92	77 ± 6*	47 ± 5	77 ± 5*	57 ± 4	75 ± 4*	32 ± 4	74 ± 6*	45 ± 4	53 ± 5*	0	62 ± 4*	9 ± 2
2.28	54 ± 3*	5 ± 1	51 ± 3	48 ± 5	56 ± 4*	3 ± 1	54 ± 3*	22 ± 3	37 ± 3*	0	46 ± 3*	0
13.5	27 ± 3*	2 ± 1	21 ± 1*	30 ± 4	38 ± 4*	0	41 ± 3*	10 ± 2	19 ± 3*	0	28 ± 3*	0
81.7	8 ± 1*	0	7 ± 2	6 ± 3	16 ± 2*	0	18 ± 2*	8 ± 1	8 ± 1*	0	11 ± 2*	0

*Significantly different from value for single spermatozoa ($p \leq 0.005$)

and single sperm were traced with the "trail-draw" facility of the Hobson Sperm Tracker and are shown in Figure 2. Paired spermatozoa maintained a forward progression at the highest viscosity. Single spermatozoa moved in circles of decreasing diameter with increasing viscosity and finally stopped moving completely above 13.5 poise viscosity. The patterns of flagellum movement of paired and single spermatozoa were observed from single-frame analysis of appropriate video recordings (Fig. 3). The flagellum of each paired spermatozoon always beats in equal but opposite synchrony with its partner (scissor action), although at high viscosities the beat frequencies were much slower.

DISCUSSION

The aim of this study was to assess the hypothesis that paired opossum spermatozoa have a motility advantage over unpaired spermatozoa and may have evolved in response to selection in the female tract [5]. Previous investigators [9, 13] found no obvious quantitative difference in the motility of single and paired opossum (*Didelphis*) spermatozoa in standard culture medium. In order to mimic the more viscous fluids that spermatozoa might encounter in the female tract, spermatozoa were incubated in solutions of increasing viscosity obtained by adding PVP to culture medium previously shown to maintain sperm motility and induce capacitation and fertilization in vitro [15]. In preliminary studies, opossum sperm maintained motility with PVP for a longer period than with other substances (Ficoll, Percoll; data not shown). Changes in motility brought about by PVP were apparently temporary (as indicated by the resumption of maximum motility of spermatozoa returned to medium without PVP), suggesting that the effects on spermatozoa were physical. Clearly, the physiochemical forces on spermatozoa in the female tract involve complex interactions with secretions and epithelial surfaces that cannot be simulated fully in vitro. Nevertheless, previous investigations have demonstrated the usefulness of viscous solutions in elucidating the function of sperm movement [19].

A direct comparison of movement parameters of single and paired spermatozoa in medium without PVP revealed that the absolute mean straight-line and curvilinear velocities of paired spermatozoa were significantly ($p < 0.05$) greater than those of single spermatozoa and that the lateral head displacement of paired sperm was about half that of single sperm. Thus, paired sperm displayed very straight progression while single sperm moved in a straight but undulating manner (Fig. 3). The relatively rapid movement of either paired or single spermatozoa in the standard (nonviscous) medium could be accurately followed by the Hobson Sperm Tracker but was very difficult to assess visually. This probably accounts for the conclusion reached by previous investigators that there was no apparent visual difference in the progressive motility of paired and single spermatozoa [9, 13]. Since single and paired spermatozoa displayed different motions, we considered that the best method of comparing the efficiency of their movement in viscous media was to calculate velocity parameters as fractions of the maximum value in medium alone. This analysis also took into account that the forces generated by single and by paired spermatozoa may not always be equivalent. Moreover, since single spermatozoa recovered from the cauda epididymidis might not have found a partner because of some functional defect, it was important to examine spermatozoa that had unpaired during incubation in vitro; therefore, various incubation times were used.

At the lowest concentration of PVP (viscosity, 0.8 poise), there was no significant difference between the movement values for single and paired spermatozoa incubated for up to 2 h when calculated as a percentage of their maximum velocities; this indicated a similar efficiency of motion. However, at higher viscosities, paired spermatozoa exhibited significantly greater progressive motility than did single spermatozoa. This was most apparent after 2-h pre-incubation; for example, at a viscosity of 2.3 poise, the progressive motility of single spermatozoon was only 3% of maximum while that of paired spermatozoa remained 56% of maximum. Interestingly, it is after about 2 h of incubation that paired spermatozoa begin to separate and will then fertilize eggs in vitro [15]. By contrast, curvilinear velocities for single and paired sperm remained relatively similar, indicating that the movement activity of single spermatozoa was equal to that of pairs. By 24 h of incubation, the velocities of both sperm types were reduced, perhaps reflecting the overall survival time of about 30 h for opossum spermatozoa in vitro. Although opossum body temperature is about 33°C when resting, previous investigations of in vitro fertilization [15] showed that capacitation and sperm unpairing occurred more effectively at 37°C in culture. In the present study, the initial motility measurements were made at 30 and 37°C to examine possible difference in motility parameters. Sperm velocities were higher at 37°C than 30°C, but the same differences between single and paired spermatozoa were observed.

Although a pair of spermatozoa have double the head size and mass of a single spermatozoon, their conjugation was clearly advantageous under viscous conditions. Why was this the case? The forward momentum of a spermatozoon is the sum of the thrust and torque developed by the undulations of the flagellum minus the passive drag and resistive movement of the overall sperm body, i.e., flagellum and head (see appendix of [19]). In each spermatozoon, some of the thrust of the flagellum will always be dissipated in movement of the head (lateral head displacement), thereby reducing the force available for propulsion and increasing drag. With paired sperm, the flagellum of each cell appeared to beat in equal but opposite synchrony with its partner as first reported by Phillips [10]. This was most apparent in high-viscosity medium, where the beat frequency was sufficiently slow to allow clear sight of the tail patterns,

or in slow-motion video recordings. Hence, the torque forces acting on one sperm head would be counterbalanced by a similar but opposite force from the partner sperm head, thereby minimizing the loss of forward propulsion and reducing drag, particularly in viscous medium. The shape of the paired sperm heads also appears to be more hydrodynamic than a single head and therefore may allow more efficient movement through medium [9].

In undertaking this work, we assumed that opossum spermatozoa would encounter an environment in the female tract that would be more viscous than in medium alone. Certainly in other marsupials a viscous vaginal mucus has been observed at the time of estrus [20, 21]. It has been suggested that this secretion may be associated with the maintenance of a sperm reservoir [21]. In *Didelphis virginiana* [13] and in *Monodelphis domestica* (Moore, unpublished observation), paired spermatozoa enter crypts in the lower isthmic region of the oviduct, where they remain surrounded by a dense epithelial secretion prior to ovulation. In preliminary experiments, we have recovered the luminal contents of the isthmic region of oviducts dissected from female opossums 8 h after mating by using a fine glass capillary tube (heparin-treated) inserted at the uterotubal junction. Although only small quantities of fluid were obtained (1–2 μl/oviduct), the resistance to flow within the capillary tube suggested that the viscosity was greater than a 5% PVP medium (13.5 poise). At this viscosity, only paired spermatozoa would have progressive motility. Exactly where paired sperm separate in the oviduct is at present unclear. In histological sections of the isthmus region recovered after mating, paired spermatozoa are observed, but only single sperm bind to the zona pellucida [12], suggesting that unpairing occurs shortly before fertilization in the ampulla region of the oviduct.

As in the Virginian opossum, *Didelphis virginiana* [4], few sperm are present in the epididymis of *Monodelphis* (*ca* 4×10^6 sperm/epididymis; [11]) relative to numbers in most other mammals, and even fewer are located in the caudal sperm storage region of the duct. Thus the number of spermatozoa available at ejaculation is very low. However, the data from *Didelphis* indicate that spermatozoa from this species, at least, are very successful in terms of the number inseminated, and about 1 in 20 reach the site of fertilization [4]. This compares with 1 in 5000–10000 spermatozoa for a typical eutherian mammal such as the rabbit [22]. Although caution is needed in extrapolating the findings in an extant species to those of ancestral species, our present results indicate that sperm pairing may have evolved as a mechanism for the efficient movement of spermatozoa reaching the isthmus region of the oviduct. The evolution of sperm pairing may have then led to a concomitant decrease in sperm production and storage since the requirement for large numbers of ejaculated spermatozoa was reduced when a relatively greater proportion reached the site of fertilization.

The exact nature of the selective pressure that would bring about sperm pairing/unpairing remains uncertain, but certain features of marsupial reproduction provide some clues. Unlike most oocytes of eutherian mammals, which are surrounded by a cumulus mass after ovulation, those of marsupials are naked. Moreover, the zona pellucida of the marsupial egg is thin in comparison to that of eutherian oocytes. Thus, the viscosity/resistance of the environment immediately surrounding the marsupial egg may be relatively low, allowing the unpaired spermatozoon sufficient motility for fertilization. This is in contrast to conditions in the hamster, for example, where spermatozoa are thought to display hyperactivated motility in order to pass through the viscous matrix of the cumulus mass surrounding the egg and the rigid zona pellucida surrounding the oolemma [19]. Another feature of reproduction in marsupial but not eutherian mammals is the formation of a mucoid coat and shell membrane surrounding the zygote as it passes down the tract. In order for these coats to be deposited, a thick viscous secretion is present in the lower isthmus of the oviduct and in the uterus. Mechanisms may have evolved, therefore, to enable spermatozoa to move efficiently in viscous fluids in response to the co-evolution of these egg coats. It is of interest that in the Australian dasyurid marsupials, low epididymal and ejaculatory sperm numbers have also been described [23], along with remarkably efficient sperm passage to the oviduct (1 in 1–7 of ejaculated spermatozoa). Although these spermatozoa do not form pairs, they display a peculiar undulating motility that also enables them to move efficiently together through viscous solutions. Thus the prime mover for evolution of efficient sperm motility may be in response to the co-evolutionary changes to protect the zygote/embryo.

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