Motility of spermatozoa in hydrosalpingeal and follicular fluid of pigs

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Hydrosalpinges were created to collect adequate volumes of fluid during pre-, peri- and postovulatory intervals from the ampulla, ampullary–isthmic junction and the isthmic–uterine junction of the oviducts from Large White gilts that had exhibited at least two natural oestrous cycles. The accumulated fluids, follicular fluid and Butschwiler's medium were compared for their effects on various parameters of boar sperm motility using the CellSoft®, computer-assisted, digital image analysis system. Sperm velocity (µm s⁻¹ ± SEM) was significantly higher (P < 0.05) in follicular fluid (84 ± 3; n = 5) than in fluids from the ampulla during per- and early postovulatory intervals, and from the isthmic–uterine–tubal junction during pre- and early postovulatory intervals. It was also higher (P < 0.05) than in the fluid from the ampullary–isthmic junction during pre- and early postovulatory intervals; however, sperm velocity in follicular fluid was not significantly different from that in the periovulatory fluid from the ampullary–isthmic junction. The mean lateral head displacement (ALH̅ mean) of spermatozoa was significantly greater in follicular fluid (3.9 ± 0.3 µm; n = 5) than in fluid from the ampulla during peri- and early postovulatory intervals and from the isthmic–uterine–tubal junction during pre- and early postovulatory intervals, and was also higher (P < 0.05) than in fluid from the ampullary–isthmic junction during the preovulatory period, but was not different from the peri- and postovulatory ampullary–isthmic junction fluids. The proportion of spermatozoa exhibiting circular motion was significantly higher (P < 0.05) in the periovulatory fluid from the ampullary–isthmic junction (24 ± 3%) compared with fluids obtained during preovulatory and early postovulatory periods. Follicular fluid had no effect on the proportion of spermatozoa exhibiting circular motion. The average radius of sperm movement in circular trajectories was higher in follicular fluid than in the periovulatory fluids from the ampulla and ampullary–isthmic junction (P < 0.05). In hydrosalpingeal fluids collected 2–5 days after ovulation, the average radius of movement was greater in the ampulla fluid and ampullary–isthmic junction fluid than in fluid from the isthmic–uterine–tubal junction (P < 0.05). These results demonstrate that follicular fluid and oviductal fluids have considerable influences on boar sperm motility. Furthermore, the immediate effect of periovulatory ampullary–isthmic junction fluid in increasing the percentage of spermatozoa swimming in circles (hyperactivated) is relevant, since it is at this time and within this region that fertilization occurs.

Introduction

The primary purpose of sperm transport is to establish a population of competent spermatozoa at or near the site of fertilization in the oviducts close to the time of ovulation, so that penetration and activation of the oocyte can occur before postovulatory ageing of the oocyte commences (Hunter, 1987; Yanagimachi, 1994). However, before they become capable of fertilizing eggs, spermatozoa must undergo a process termed capacitation. The site in the female reproductive tract where this begins is unknown and may vary from species to species (for review, see Yanagimachi, 1994) but capacitation occurs more rapidly if the spermatozoa are sequentially exposed to the uterus and then the oviductal environment (Hunter and Hall, 1974). The process involves membranous reorganization...
that permits and culminates in changes in motility, termed hyperactivation, and in the acrosome reaction.

Spermatozoa change their motility pattern depending on the physical and chemical characteristics of the surrounding medium. The pattern of hyperactivation is influenced greatly by the surrounding microstructure and the viscosity of the medium (Yanagimachi, 1994). To this end, and related to the finite lifespans of the gametes, the oviduct has evolved certain physiological strategies to regulate the passage of spermatozoa, which involve the selection, storage, maturation and activation of an elite population of competent spermatozoa to fertilize the egg.

Assuming that mating takes place at the beginning of standing oestrus, viable spermatozoa can be retained in the isthmus in the pig for 36 h or more (Hunter, 1987). Their immobilization in this region may be due to diverse factors that probably act in combination: their movement may be restricted by the abundant secretion of mucus found in the isthmus and utero–tubal junction before ovulation (Jansen and Baipai, 1982); the lower temperature in the isthmus of the pig oviduct (Hunter and Nichol, 1986); and fluid composition, especially the concentrations of ions, energy substrates and hormones (Ericsson et al., 1967; Burkman et al., 1984).

The initial passage of spermatozoa from the isthmus to the ampulla is closely synchronous with ovulation (Hunter, 1987). This is related to the maturity of the largest Graafian follicles, which cause steroids and prostaglandins to be released into the ovarian vein. These hormones enter the ovarian and utero–tubal arteries by a local counter-current transfer, changing the patency and contractile activity of the isthmus (Hunter et al., 1983). These hormones may also enter the oviducal fluid in the isthmic region and stimulate the motility of the spermatozoa directly (Ericsson et al., 1967).

Spermatozoa, now liberated, ascend the oviduct in finely regulated numbers to the site of fertilization in the ampullary–isthmic junction (Hunter, 1987). Only a very small fraction of the spermatozoa in the isthmus continue their migration; of the thousands that reach the isthmus, only 2–20 reach the ampulla at any one time (Suarez et al., 1990). Their ascent is marked by a slow progression through the isthmus: the spermatozoa appear to become attached to the oviducal wall for a time; they then break free to swim for a distance; they then become attached again (Suarez et al., 1990). These facts imply that some form of interaction or exchange is occurring between the spermatozoa and the epithelium.

In rabbits, all the spermatozoa found in the isthmus are quiescent, even though they exhibit hyperactivated motility in the ampulla (Overstreet and Cooper, 1975). Uncapacitated spermatozoa are relatively inflexible, particularly in the region of the mid-piece, although the tip of the tail can flex. In contrast, capacitated spermatozoa have fluid-like movements that are both rapid and contorted, often described as 'whiplash movement' (Suarez et al., 1990, 1992).

The specific objective of these studies was to characterize the types of movement of boar spermatozoa when they are swimming in hydrosalpingeal fluid obtained from different regions of the oviduct at different stages of the oestrous cycle, in pig follicular fluid and in buffer.

Materials and Methods

Animals

Twenty Large White gilts, 9–24 months old, were penned indoors in groups of five or six under a natural lighting regime. They were fed twice a day on a normal commercial diet with free access to water. They had exhibited at least two spontaneous oestrous cycles before the experiments began, verified by daily heat checks.

Surgery and fluid sampling

Animals were fasted for 24 h before surgery. Anaesthesia was induced by an intravenous injection of sodium pentobarbital (Somnotol, CDMV Inc., St Hyacinthe, Quebec), the dose being sufficient to permit endotracheal intubation. Anaesthesia was thereafter maintained on a closed-circuit apparatus using halothane (Fluothane, ICI Pharmaceuticals, distributed by CDMV Inc.), nitrous oxide and oxygen. Strict aseptic techniques were used since the reproductive tract was exposed by a mid-ventral incision. The oviducts were gently exteriorized, one at a time, and ligated into sections approximately 3 cm long. Three compartments were ligated, each representing a different region of the oviduct: (1) the ampulla; (2) the ampullary–isthmic junction; and (3) the isthmic–utero–tubal junction. Manipulation of the tissues was kept to a minimum to avoid trauma, and the bulk of the genital tract was left within the abdomen to prevent dehydration during the surgical procedures. Animals were slaughtered 1–5 days later, after which the reproductive tract was removed and immediately transported on ice to the laboratory. It was necessary to examine the ligated areas of the oviduct for visible signs of inflammation and the samples from the hydrosalpinges for possible blood contamination because there are many compounds, including prostaglandins (Hunter, 1987) and progesterone (Hansen et al., 1989), that affect sperm motility and capacitation. Although these occur naturally in follicular fluid, any signs of inflammation or blood contamination of the hydrosalpingeal fluids would be considered abnormal and would therefore preclude the use of these fluids in our studies. Gross examination revealed no evidence of inflammation of the tissues around the ligatures and, since all procedures were done by the same operator, any invisible inflammatory response was considered to be constant for all samples. The fluid that had accumulated in the different regions of the oviducts was aspirated into 1 ml syringes and stored immediately at −80°C to minimize degradation of sample components by contact with air. Any samples that were discoloured or that may have been contaminated with blood were not used (David et al., 1969, 1973). Surgery was performed according to the following timetable. Preovulatory (pre-) hydrosalpingeal fluid was obtained from animals that were both ligated and slaughtered before ovulation. Periovulatory hydrosalpingeal fluid (peri-) was obtained from animals ligated 2 days before ovulation and slaughtered 1 day after ovulation. Early post-ovulatory (post-1) hydrosalpingeal fluid was obtained from animals ligated 1 day after ovulation and slaughtered 4, 5 or 6 days after ovulation. Later postovulatory (post-2) hydrosalpingeal fluids were obtained from animals ligated 2 days
after ovulation and slaughtered 5 or 6 days after ovulation. Pools of follicular fluid were obtained from individual ovaries by combining the fluid from the large preovulatory follicles (approximately 8 mm in diameter) from five control pigs that were ovariotomized as part of a separate study. The follicular fluid was immediately frozen and stored at −20°C before use.

Semen collection and sample preparation

Semen was obtained from a mature Large White boar by the manual method over a dummy sow (Glover, 1955; Polge, 1956). The boar was approximately 2 years old, had been used as a breeder and was of proven fertility. This was further verified in our laboratory by the development of embryos flushed from the oviducts of some animals that had been mated to this boar. A single boar was used in these studies to reduce costs and animal-to-animal variation in the sperm motility analysis. The sperm-rich fraction of the ejaculate was directed into a sterile container that had been kept at body temperature until the time of collection and that was then allowed to cool to room temperature (18°C). Immediately thereafter, the semen was diluted (1:5) in Butschwiler’s medium (195 mmol glucose 1⁻¹, 23 mmol sodium citrate 1⁻¹, 12 mmol sodium bicarbonate 1⁻¹, 7 mmol EDTA (disodium salt) 1⁻¹, 15 mmol citric acid 1⁻¹, 47 mmol Tris 1⁻¹, 0.45 mmol cysteine 1⁻¹, 3.0 g BSA 1⁻¹, pH 6.92, 373 mOsm) and maintained at 18°C during transport to the laboratory (40 min). A population of motile spermatozoa was then isolated by Percoll density gradient centrifugation (80%, 60%, 40% Percoll layers buffered with Butschwiler’s medium, pH 6.92, 373 mOsm; 1100 g for 30 min). Purified spermatozoa (10 µl, collected from the bottom of the 80% Percoll layer) were added to 90 µl test sample; 8 µl was then placed in a Makler chamber 10 µm deep (Sefi-Medical Instruments, Haifa) on the heated stage (37°C) (Microwarm plate 30 Kita Zato, Japan) of the microscope. Sperm motility analysis was performed immediately. The concentration of spermatozoa was between 25 and 40 × 10⁶ ml⁻¹, which was sufficient to obtain reliable statistical data and averted the problems associated with high concentrations (where spermatozoa may come into contact with each other, rendering analysis impossible) and with low concentrations (where the analysis would take too long to monitor sufficient numbers of spermatozoa to obtain meaningful data). With the concentrations used, it was possible to analyse the effects of hydrosalpingeal samples and follicular fluid on sperm motility within a few minutes.

Sperm motility analysis

Computer-assisted sperm motility analysis was performed using the 'CellSoft®' system (Montgomery, NY). Each hydrosalpinx was assayed once with the following set-up parameters: the number of frames to be analysed was 20, at 30 frames per second; the minimum number of samples for motility and velocity was three and ten, respectively; the minimum and maximum velocities were 10 and 250 µm s⁻¹, respectively; the pixel scale was 1.72 µm per pixel (magnification × 26.8); the cell size range was 3–100 pixels; and the maximum radius for circular motion was 150 µm.

These settings were used to quantify the following sperm motility parameters for each sample of hydrosalpingeal fluid, follicular fluid or buffer: (1) percentage of motile spermatozoa; (2) curvilinear velocity (µm s⁻¹); (3) linearity; (4) mean lateral head displacement (ALHmean, µm); (5) percentage of circling over motile cells; and (6) average radius of sperm motion (µm). A minimum of 200 spermatozoa were analysed from at least five different fields that were recorded on the videotape.

Statistical analysis

Motility parameters were analysed by one-way ANOVA but multiple range tests could not compare all groups because of the unequal numbers in groups. Therefore, where ANOVA probabilities were P < 0.05, significantly different groups were detected by Student’s t test (Sigmapstat Programme Version 1 for Windows, Jandel Scientific, 1992–1994, San Rafael, CA). Comparisons were made between fluids (ampulla, ampullary–isthmic junction, isthmic–isthmic–utero–tubal junction, follicular fluid and buffer) and between stages of cycle (pre-, peri-, post-1 and post-2). In five animals, hydrosalpingeal fluid was obtained from both left and right oviducts. Comparisons of sperm motility parameters in hydrosalpingeal fluids obtained from these five animals showed no significant effect of left or right side (P > 0.05); therefore, due to the unequal distribution of follicles between ovaries, all fluid samples were considered as coming from individuals.

Results

The effects of hydrosalpingeal and follicular fluid on the velocity, lateral head displacement, the average radius of sperm motion and the proportion of spermatozoa exhibiting circular motion are shown (Figs 1–4). Sperm velocity was significantly higher (P < 0.05) when spermatozoa were added to follicular fluid (84 ± 3.02 µm s⁻¹; mean ± SEM) compared with the periovulatory and post-1 fluids from the ampulla (58.7 ± 2.1 and 61.7 ± 4.1 µm s⁻¹, respectively), and was also higher compared with the preovulatory and post-1 fluids from the ampullary–isthmic junction (64.8 ± 3.2 and 63.8 ± 4.8 µm s⁻¹, respectively) and the isthmic–isthmic–utero–tubal junction (53.0 ± 6.0 and 53.3 ± 3.7 µm s⁻¹, respectively). However, the velocity in follicular fluid, although higher than that in periovulatory ampulla fluid (P < 0.05), was not significantly different from the velocity in periovulatory fluid from the ampullary–isthmic junction (Fig. 1). In hydrosalpingeal fluids collected from the ampulla 2–5 days after ovulation (post-2), spermatozoa exhibited a velocity (77 ± 2.6 µm s⁻¹) that was higher than that observed in periovulatory ampulla but was similar to that observed in follicular fluid. In fact, sperm velocity in post-2 fluids from all three regions of the oviduct was not different from that in follicular fluid (Fig. 1).

The increased velocity of spermatozoa in follicular fluid was accompanied by a significant increase in the ALHmean (Fig. 2) compared with spermatozoa observed in the hydrosalpingeal periovulatory and post-1 fluids from ampullae. Similarly, the ALHmean was significantly greater in follicular fluid compared with the fluid from the preovulatory ampullary–isthmic junction and the preovulatory and post-1 fluids from the isthmic–isthmic–utero–tubal junction (P < 0.05). However, the ALHmean
Fig. 1. The velocity of boar spermatozoa was measured in hydrosalpingeal fluid obtained from (a) the ampulla, (b) the ampullary–isthmic junction and (c) the isthmic–utero–tubal junction of pig oviducts, according to the schedule described in the Materials and Methods section. Values are means ± SEM and numbers within bars represent the number of samples tested. Dissimilar superscripts indicate significant differences between fluids (P < 0.05). Bars with no superscripts represent groups where n < 3; these groups were not included in the statistical analysis and are presented for comparative purposes only. Pre-, preovulatory fluid obtained from animals both ligated and slaughtered before ovulation; Peri-, periovulatory fluid obtained from animals ligated 2 days before ovulation and slaughtered 1 day after ovulation; Post-1, early postovulatory fluid obtained from animals ligated 1 day after ovulation and slaughtered 4, 5 or 6 days after ovulation; Post-2, later postovulatory fluid obtained from animals ligated 2 days after ovulation and slaughtered 5 or 6 days after ovulation; FF, pig follicular fluid; buffer, Butschwiler’s medium.

Fig. 2. The mean lateral head displacement ($\text{ALH}_{\text{mean}}$) of boar spermatozoa was measured in hydrosalpingeal fluid obtained from (a) the ampulla, (b) the ampullary–isthmic junction and (c) the isthmic–utero–tubal junction of pig oviducts, according to the schedule described in the Materials and Methods section. Values are means ± SEM and numbers within bars represent the number of samples tested. Dissimilar superscripts indicate significant differences between fluids (P < 0.05). Bars with no superscripts represent groups where n < 3; these groups were not included in the statistical analysis and are presented for comparative purposes only. Pre-, preovulatory fluid obtained from animals both ligated and slaughtered before ovulation; Peri-, periovulatory fluid obtained from animals ligated 2 days before ovulation and slaughtered 1 day after ovulation; Post-1, early postovulatory fluid obtained from animals ligated 2 days before ovulation and slaughtered 4, 5 or 6 days after ovulation; Post-2, later postovulatory fluid obtained from animals ligated 2 days after ovulation and slaughtered 5 or 6 days after ovulation; FF, pig follicular fluid; buffer, Butschwiler’s medium.
Table 1. Boar sperm motility characteristics in hydrosalpingeal fluids from pig oviducts obtained at different stages of the oestrous cycle and in porcine follicular fluid

<table>
<thead>
<tr>
<th>Region</th>
<th>Motile spermatozoa (%)</th>
<th>Linearity</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM ($n$)</td>
<td>Mean ± SEM ($n$)</td>
</tr>
<tr>
<td>Ampulla</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preovulatory</td>
<td>54 ± 7 (2)</td>
<td>5.7 ± 0.4 (2)</td>
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<tr>
<td>Periovulatory</td>
<td>40 ± 5 (10)</td>
<td>3.8 ± 0.2 (10)</td>
</tr>
<tr>
<td>Postovulatory-1</td>
<td>41 ± 7 (13)</td>
<td>4.1 ± 0.3 (12)</td>
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<tr>
<td>Postovulatory-2</td>
<td>62 ± 5 (4)</td>
<td>3.6 ± 0.3 (4)</td>
</tr>
<tr>
<td>Ampullary-isthmic junction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preovulatory</td>
<td>41 ± 7 (4)</td>
<td>3.9 ± 0.2 (4)</td>
</tr>
<tr>
<td>Periovulatory</td>
<td>44 ± 10 (8)</td>
<td>3.4 ± 0.6 (8)</td>
</tr>
<tr>
<td>Postovulatory-1</td>
<td>41 ± 8 (11)</td>
<td>3.8 ± 0.2 (13)</td>
</tr>
<tr>
<td>Postovulatory-2</td>
<td>48 ± 7 (5)</td>
<td>3.5 ± 0.1 (5)</td>
</tr>
<tr>
<td>Isthmic–utero–tubal junction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preovulatory</td>
<td>29 ± 8 (3)</td>
<td>4.3 ± 0.4 (3)</td>
</tr>
<tr>
<td>Periovulatory</td>
<td>22 ± 1 (1)</td>
<td>5.8 ± 1</td>
</tr>
<tr>
<td>Postovulatory-1</td>
<td>38 ± 10 (5)</td>
<td>3.8 ± 0.4 (6)</td>
</tr>
<tr>
<td>Postovulatory-2</td>
<td>49 ± 9 (3)</td>
<td>4.4 ± 0.2 (2)</td>
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<tr>
<td>Follicular fluid</td>
<td>57 ± 6 (5)</td>
<td>4.5 ± 0.4 (5)</td>
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<tr>
<td>Buffer</td>
<td>56 ± 4 (3)</td>
<td>4.0 ± 0.3 (3)</td>
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Boar sperm motility characteristics were monitored in hydrosalpingeal fluids obtained from the ampulla, ampullary–isthmic junction and the isthmic–utero–tubal junction from pig oviducts at different times of the oestrous cycle, in porcine follicular fluid obtained from preovulatory follicles and in Butschwiler’s medium (buffer). No significant differences were found ($P > 0.05$).

in post-2 fluids from all three regions of the oviduct was not significantly different compared with follicular fluid ($P > 0.05$), and this was also the case with periovulatory and post-1 fluids from the ampullary–isthmic junction ($P > 0.05$).

Although follicular fluid caused a more vigorous motion of spermatozoa, the percentage of motile spermatozoa was unaltered, as was the linearity (Table 1). However, the proportion of motile spermatozoa exhibiting circular motion differed in the different fluids: it was significantly higher in periovulatory fluid from the ampullary–isthmic junction compared with follicular fluid and preovulatory and post-1 hydrosalpingeal fluids collected from the ampullary–isthmic junction but was not significantly higher than that in post-2 hydrosalpingeal fluids ($P = 0.055$). Furthermore, the proportion of motile spermatozoa exhibiting circular motion was significantly higher in periovulatory fluid from the ampullary–isthmic junction compared with periovulatory fluid from the ampulla (24.3 ± 3.0% versus 16.3 ± 2.3%; $P < 0.05$) (Fig. 3).

The average radius of the sperm motion in follicular fluid was significantly greater than that observed in periovulatory hydrosalpingeal fluids from the ampulla and in preovulatory, periovulatory and post-1 fluids from the ampullary–isthmic junction ($P < 0.05$) (Fig. 4) but was not significantly higher than that in post-2 fluid.

Discussion

During capacitation, spermatozoa undergo a change in the pattern of their motility before fertilization of the ovum, in that...
they assume a more vigorous and circular swimming motion called hyperactivation (Suarez et al., 1992; Yanagimachi, 1994). The present investigations were designed to determine whether fluid collected from different regions of the oviduct and at different stages of the pig oestrous cycle had specific consequences for sperm motility characteristics. We opted to collect hydrosalpingeal fluids as opposed to single point samples of oviductal fluid because the volume of sample collected was greater with hydrosalpinges. We have developed a method for obtaining pure oviductal fluids but the volumes obtained are very small (4–8 µl) (Nichol et al., 1992); for the studies reported here, such small volumes would have been insufficient. Repeated operations would have been required to obtain sufficient amounts of fluid by this method and this would have put undue stress on the animals and would also have contravened the guidelines of the Canadian Council on Animal Care, to which all procedures conformed.

Overall, the effects on spermatozoa of hydrosalpingeal fluid collected from pigs in different reproductive states were similar, regardless of the region of the oviduct from which the fluids were obtained. The major difference was that the fluid obtained from the ampullary–isthmic junction during the periovulatory period increased the proportion of spermatozoa exhibiting circular motion. The circular motion of boar spermatozoa hyperactivated with the calcium ionophore has previously been described by Suarez et al. (1992), who observed a small proportion of hyperactivated spermatozoa in flushings of gilt oviducts. It is possible that the proportion was small because the biological fluids were diluted at the time of collection. For our studies, we decided that, to obtain an appreciable quantity of fluid without the necessity for dilution, ligation of the oviducts offered the best solution. Our finding that the percentage of spermatozoa swimming in circles in fluid from the ampullary–isthmic junction during the periovulatory period was almost as high as that obtained with the calcium ionophore (30%) (Suarez et al., 1992) lends some justification for the chosen technique. The occurrence of sperm hyperactivation in fluid from the ampullary–isthmic junction during the periovulatory period may be of considerable significance because it is at this time and within this region that fertilization of pig oocytes occurs (Hunter, 1974). Thus, there appear to be components in the ampullary–isthmic junction fluid that stimulate circular motion around the time when eggs will be present. This effect was not seen in preovulatory or early postovulatory fluids and, although not significant, the proportion of sperm exhibiting circular motion in periovulatory fluid was higher than in later postovulatory fluid from the ampullary–isthmic junction (post-2). Of further significance was the time frame within which the increase in circular motion was observed in oviductal fluid. Our analyses were performed immediately after the spermatozoa and oviducal fluid were combined; the circling motion was observed within minutes. In synthetic media, mouse sperm hyperactivation occurs after incubation for approximately 1 h (Neill and Olds-Clarke, 1987) between 1 and 3 h for humans (Burkman, 1990) and after 5 h for hamsters (Shalgi et al., 1992).

The second interesting finding is that the fluid that influenced sperm motility most significantly was follicular fluid. Increased velocity and lateral head displacement were indicative of a more vigorous motion, and the higher radial curvature

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**Fig. 4.** The average radius of motion of boar spermatozoa was measured in hydrosalpingeal fluid obtained from (a) the ampulla, (b) the ampullary–isthmic junction and (c) the isthmic–uto–tubal junction of pig oviducts, according to the schedule described in the Materials and Methods section. Values are means ± SEM and numbers within bars represent the number of samples tested. Dissimilar superscripts indicate significant differences (P < 0.05). Bars with no superscripts represent groups where n < 3; these groups were not included in the statistical analysis and are presented for comparative purposes only. Pre-, preovulatory fluid obtained from animals both ligated and slaughtered before ovulation; Peri-, periovulatory fluid obtained from animals ligated 2 days before ovulation and slaughtered 1 day after ovulation; Post-1, early postovulatory fluid obtained from animals ligated 1 day after ovulation and slaughtered 4, 5 or 6 days after ovulation; Post-2, late postovulatory fluid obtained from animals ligated 2 days after ovulation and slaughtered 5 or 6 days after ovulation; FF, pig follicular fluid; Buffer, Butschwilker’s medium.
also observed may have been a consequence of the increased rapidity of forward movement in the absence of a stimulator of curved motility. In this regard, it is worth noting that the average radius of sperm trajectories was smaller in the peri-ovulatory fluid from the ampullary–isthmic junction than in follicular fluid, as it was in the postovulatory fluids collected from all regions of the oviduct. Consequently, it is possible that fluids from the oviduct and follicular fluid may be acting in concert to stimulate the vigour and the direction of sperm motion, and that the combination of these enhances fertilization.

The possibility of a direct role for follicular fluid in the fertilization process in pigs has not received serious consideration since, based on progesterone measurements of oviductal fluid, it has been estimated that less than 1% of the follicular fluid is retained in the oviduct after ovulation (Hansen et al., 1989). However, R. Nichol et al. (unpublished) have observed that immediately after ovulation the pH of the oviductal fluid becomes transiently but markedly alkaline as the oocytes traverse the ampulla. These fluctuations are attributed to the passage of follicular fluid with the oocytes. In the light of these findings, a more important role for follicular fluid in the events of fertilization should be considered; this idea is supported by the fact that media of pH 8 or greater can stimulate hyperactivation of human spermatozoa (Burkman, 1990).

The site where sperm hyperactivation occurs varies between species. In the hamster and mouse, it begins shortly before spermatozoa are released from the isthmus; if hyperactivated spermatozoa are placed in the uterus, they enter the oviducts with difficulty (Shalgi et al., 1992). This is probably a reflection of the very different swimming behaviour of spermatozoa before and after hyperactivation. Before hyperactivation, sperm progression is linear (Suarez et al., 1992; Yanagimachi, 1994) but hyperactivation confers a mechanical advantage upon spermatozoa when they encounter viscous surroundings such as oviductal fluid or cumulus oophorbus matrix.

The change in sperm ‘behaviour’ seen in the region of the ampullary–isthmic junction is conspicuous. In many species (for example, mice, hamsters, rabbits), the leathargic stage of spermatozoa and their affinity for contact with the epithelium that is demonstrated in the lower isthmus changes suddenly when they enter the ampullary–isthmic junction and ampulla so that they become liberated, hyperactive and free-swimming (Overstreet and Cooper, 1975; DeV Mott and Suarez, 1992; Yanagimachi, 1994). Furthermore, a significantly higher number of free spermatozoa are present in the ampulla than in the isthmus and it appears that only those spermatozoa that are hyperactivated are able to break free from the epithelium (DeV Mott and Suarez, 1992). There appear to be no differences in sperm binding affinity between regions, at least in vitro. However, it has been shown that there is a difference between the binding of capacitated versus non-capacitated bull spermatozoa (Lefebvre and Suarez, 1995): capacitation appeared to be associated with the loss of ability to bind to the oviductal epithelium and the authors suggested that this may contribute to the release of spermatozoa from the isthmic reservoir.

A major function of the oviducal fluids is the provision of energy sources appropriate for hyperactive movement. In support of this, it has been shown that fluid from oestrous farm animals causes maximum stimulation of sperm respiration and glycolysis (Stone and Hamner, 1975). Glycolytic metabolism, and especially the presence of glucose and pyruvate, is required to support the development of the whip pattern of motility associated with the capacitation of spermatozoa of many species (Yanagimachi, 1994). Of relevance to the present studies was the finding that the concentration of glucose was higher in fluid from the ampullary–isthmic junction of pre-ovulatory pigs compared with postovulatory pigs (Nichol et al., 1992).

The pH of the surrounding medium also has regulatory consequences for sperm motility. The bicarbonate ion concentration appears to be important for capacitation, hyperactivation, acrosome reaction and zona pellucida penetration (Boatman and Robbins, 1991). Boar spermatozoa possess a bicarbonate transporter system (Okamura et al., 1988). Bicarbonate may stimulate the activation of adenyl cyclase, which produces cAMP that in turn is involved in sperm motility (Okamura et al., 1985). In the present study, any effects of pH differences between the hydrosalpingeal fluids on sperm motility analysis are likely to have been minimal since the fluids were exposed to the atmosphere, albeit for a very brief period, at the time of sampling. This exposure would have permitted some gaseous exchange that would have led to acidification. In addition, at the time of sperm analysis, the sperm samples were suspended in buffer, which was then added to the hydrosalpingeal fluids. Recently, we have recorded the fluctuations in pH within the oviductal lumen and found that the pH of postovulatory fluid from the ampullary–isthmic junction appears to be more stable compared with the preovulatory fluid from the ampullary–isthmic junction. Before ovulation, the pH of ampullary–isthmic junction fluid can fluctuate by as much as 0.5 pH units within a few minutes, whereas after ovulation, the fluctuations are significantly smaller (< 0.1 pH units) (R. Nichol et al., unpublished). Furthermore, the pH decreases between the preovulatory and postovulatory stages of the oestrous cycle (R. Nichol et al., unpublished).

In conclusion, our data are the first to show that oviducal fluids collected during defined periods of the pig oestrous cycle and from different regions of the oviduct are stimulatory to specific features of sperm motility. Follicular fluid increases sperm velocity and lateral head displacement, and oviducal fluid from the periovulatory ampullary–isthmic junction increases the proportion of spermatozoa exhibiting the circular motion that is characteristic of hyperactivated motility. The possibility that the different fluids can combine to produce hyperactivation of spermatozoa at the right place and time is intriguing and deserves further investigation.

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