

Effect of bovine ampullary and isthmic oviductal fluid on motility, acrosome reaction and fertility of bull spermatozoa

A. A. Grippo, A. L. Way and G. J. Killian*

Dairy Breeding Research Center, Department of Dairy and Animal Science, The Pennsylvania State University, University Park, PA 16802, USA

Motility, acrosome reaction and oocyte fertilizing ability were assessed for bull spermatozoa after incubation in regional (isthmic or ampullary), bovine oviductal fluid, pooled by stage of the oestrous cycle. Oviductal fluids collected daily from isthmic and ampullary cannulae implanted in the same oviduct were divided into pools, representing two oestrous cycle stages, based on daily serum progesterone concentrations. Ejaculated bull spermatozoa were incubated for 0–6 h in each type of oviductal fluid. Incubation in isthmic oviductal fluid collected during the nonluteal stage, including oestrus and ovulation, decreased overall sperm motility (from 71.7% motile spermatozoa to 34.0%) and both path ($78 \mu\text{m s}^{-1}$ versus $86\text{--}89 \mu\text{m s}^{-1}$) and progressive ($74 \mu\text{m s}^{-1}$ versus $83\text{--}85 \mu\text{m s}^{-1}$) velocities of spermatozoa motion. Spermatozoa incubated in isthmic, non-luteal oviductal fluid had a higher rate and extent of sperm acrosome reaction (213% of control versus 136–161% of control by 2 h incubation) compared with spermatozoa incubated in other oviductal fluid types. However, incubation in nonluteal ampullary fluid increased the number of spermatozoa, which were both acrosome reacted and live, and able to fertilize bovine ova (88.7% fertilized versus 75–81%). Glycosaminoglycan concentrations were similar among types of oviductal fluid ($0.77\text{--}0.88 \text{ mg ml}^{-1}$). These findings indicate that oviductal fluid differentially affects sperm function, depending on the oviduct region and the stage of the oestrous cycle at which the fluid was obtained.

Introduction

The mammalian oviduct provides the environment for gamete transport and for events leading to fertilization. The epithelium lining the oviduct and its secretory products appear to influence the functions of spermatozoa, ova and embryos. Oviductal fluid contained within the lumen is a product of both serum transudate and active secretion from the epithelium (Leese, 1988). It is well documented that secretions of oviduct epithelial cells differ with the stage of the oestrous cycle (Mastroianni *et al.*, 1961; Restall, 1966; Malayer *et al.*, 1988; Buih *et al.*, 1989; Killian *et al.*, 1989; Verhage *et al.*, 1989; Boice *et al.*, 1990; Gerena and Killian, 1990; Grippo *et al.*, 1992). Because the composition of oviductal fluid collected at different stages of the oestrous cycle varies, it is likely that its effects on the gametes vary during the course of the cycle.

There is also evidence that the effects of the oviduct on sperm function differ with oviductal region. The oviductal isthmus may serve as a sperm reservoir, where it is likely that capacitation occurs (First and Parrish, 1987). In cattle, spermatozoa are retained in the isthmus for up to 20 h (Hunter and Wilmut, 1984). Because the ampulla is the likely site of fertilization, spermatozoa in the ampulla must be both capacitated and induced to undergo the acrosome reaction to penetrate the ovum.

Despite the potential importance of the oviduct in determining the success of fertilization, relatively little is known of how it affects sperm function, and how its effects may vary with oviduct region and stage of the oestrous cycle. A modification of surgical methods developed in our laboratory (Kavanaugh and Killian, 1988; Kavanaugh *et al.*, 1992) has enabled collection of oviductal fluid from both the isthmus and ampulla of the same oviduct. The goal of this study was to compare the effects of fluids collected from different oviduct regions and oestrous cycle stages on bull sperm motility, acrosome reaction and the ability of spermatozoa to fertilize bovine oocytes *in vivo*.

Materials and Methods

Oviductal fluid collection and storage

Fluid was collected from cannulated oviducts of ten cows as described by Kavanaugh and Killian (1988) and Kavanaugh *et al.* (1992). Fluid was collected from both the isthmus and ampulla during a single cycle of each cow, and daily samples were combined into nonluteal or luteal pools. Stages of the oestrous cycle were defined by serum progesterone concentrations determined by radioimmunoassay on daily serum samples as described by Killian *et al.* (1989). The luteal stage consisted of days when serum progesterone was $\geq 1.5 \text{ ng ml}^{-1}$. The nonluteal stage comprised days of oestrus and

*Correspondence and reprint requests.
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ovulation and the two subsequent days. Oestrus was assumed to be 4 days before the initial rise in serum progesterone, following a period when progesterone concentrations were $< 1.5 \text{ ng ml}^{-1}$. Ovulation was assumed to occur on the day after oestrus. To support comparisons of oviductal fluid from clearly defined luteal with that from ovulatory stages, oviductal fluid collected when serum progesterone was $< 1.5 \text{ ng ml}^{-1}$, but before the day of ovulation, was not analysed. Stages were assigned on the basis of observations of behavioural oestrus made in the original study (Killian *et al.*, 1989). The osmolality of oviductal fluid for nonluteal and luteal pools was: 269.2 ± 11.3 (ampullary, nonluteal); 265.3 ± 8.7 (ampullary, luteal); 267.7 ± 8.7 (isthmic, nonluteal); and 263.5 ± 46.1 (isthmic, luteal).

Procedures for assessing sperm function

Collection of spermatozoa. Modifications of the method of Parrish *et al.* (1988), as described by McNutt and Killian (1991), were used to determine the effect of oviductal fluid on sperm motility, viability, capacitation and the acrosome reaction. Briefly, ejaculated semen was collected from three bulls previously selected for similar rates of acrosome reaction in response to heparin capacitation and acrosome reaction induction with lysophosphatidylcholine (Parrish *et al.*, 1988). Spermatozoa were washed twice by centrifugation (10 min, 500 g) in protein-free, modified Tyrode's medium containing 1 mg polyvinyl alcohol ml^{-1} (Bavister, 1981; Parrish *et al.*, 1988), and the sperm pellet was diluted with modified Tyrode's medium to its original volume. Concurrently, incubation tubes were prepared with 20% oviductal fluid/80% modified Tyrode's medium (v/v) or 40% oviductal fluid/60% modified Tyrode's medium (v/v), and equilibrated in a 5% CO_2 humidified incubator at 39°C for approximately 30 min. Preliminary experiments showed that concentrations of oviductal fluid higher than 40% resulted in background staining too intense to assess acrosome reaction accurately. Spermatozoa were also incubated in heparin ($10 \mu\text{g ml}^{-1}$) and modified Tyrode's medium alone (blank controls). Controls confirmed day to day replicability, and substantiated the ability of the spermatozoa to undergo capacitation and the acrosome reaction. Three replicate experiments were performed using spermatozoa from 12 bulls and oviductal fluid from ten cows.

Percentage motility, acrosome reaction and viability. An aliquot of sperm suspension was added to each incubation tube containing oviductal fluid or control medium to yield a final concentration of 50×10^6 spermatozoa ml^{-1} , and incubated at 39°C under 5% CO_2 in air. At 0, 2, 4 and 6 h, the incubation tubes were gently but thoroughly mixed, and a slide was prepared to determine the percentage of motile spermatozoa by subjective evaluation of four fields under a microscope. The percentage of acrosome reacted spermatozoa in the first two replicates was assessed by smearing an aliquot from each reaction tube on a slide and drying and staining it with naphthol yellow–erythrosin B (Lenz *et al.*, 1982) and the number of acrosome reacted spermatozoa per 100 spermatozoa was counted. Eosin B–aniline blue stain in phosphate buffer was used to determine the viability of each sperm sample in the third replicate experiment (Shaffer and Almquist, 1948). An

aliquot of 5 μl was removed from each tube to a slide, mixed with stain, smeared and dried under a warm air current. Overall viability was determined by the number of live spermatozoa per 100 spermatozoa counted. The viability of acrosome-reacted spermatozoa, defined as the number of live or number of dead acrosome-reacted spermatozoa per 100 counted, was also assessed by eosin B–aniline blue staining (Way *et al.*, 1995). No significant differences were found between the naphthol yellow–erythrosin B and eosin B–aniline blue stains for acrosome-reacted populations of ejaculated spermatozoa capacitated with heparin (Way *et al.*, 1995). At 0, 2, 4 and 6 h after addition of spermatozoa, pH remained at 7.4–7.6 for all 40% nonluteal oviduct fluid incubation tubes.

Analysis of sperm motility. A sample from each incubation tube containing spermatozoa from two of the bulls incubated in luteal or nonluteal oviductal fluid from three cows was subjected to motion analysis. At the initial time point, these aliquots were diluted to 12.5×10^6 spermatozoa ml^{-1} , and 4 μl was pipetted onto each of three warmed Helber chambers (C. A. Hausser and Sons, Philadelphia, PA). Start times were staggered, allowing time for dilution, slide preparation and videotaping, to maintain a true zero time for each sample, and assessments were made at 0, 2 and 4 h. Videotapes of spermatozoa on each Helber chamber were prepared as described by McNutt *et al.*, 1994, and analysed by a Hamilton Thorn motion analyser (Hamilton Thorn Research Inc., Danvers, MA), connected to a Panasonic 3000 video recorder. From one to 20 spermatozoa were analysed per field scanned, with each scan set to acquire 20 sequential frames at 30 s^{-1} . Spermatozoa that collided or were agglutinated were not included in the analysis. The total number of spermatozoa analysed per treatment (two bulls \times three cows) ranged from 67 to 361. Fewer spermatozoa were analysed at 4 h because of agglutination.

Calculations of sperm movement by the motion analyser were based on the cell centre of brightness of each frame, which was used to measure several motion parameters, ALH, BCF, VSL, VCL, VAP, STR and LIN, which are defined in Table 1.

In vitro fertilization techniques

Bovine *in vitro* fertilization techniques were based on those reported by Parrish *et al.* (1988) and Bavister *et al.* (1983). Collection of ovaries, ova recovery and fertilization procedures are described by McNutt and Killian (1991). Briefly, oocytes were collected by syringe aspiration from ovaries obtained from an abattoir. Aspirated oocytes and follicular fluid were collected in 15 ml conical tubes, and allowed to settle for 30 min. Under a microscope, oocytes with an intact, compact cumulus were selected and washed three times in low-bicarbonate-Hepes medium. Cumulus–oocyte complexes were matured *in vitro* in groups of 10–15 per 50 μl drop of maturation medium (TCM-199 medium supplemented with LH, FSH, oestradiol, heat-inactivated fetal calf serum, pyruvate and gentamycin). Mature oocytes, with expanded cumulus, evenly granulated cytoplasm and apparent polar body, were observed after 22–24 h incubation at 39°C in a 5% CO_2 humidified incubator.

Table 1. Definitions of motility parameters of the Hamilton Thorn Motion Analyser

Abbreviation	Parameter	Units	Definition
ALH	Lateral head displacement	μm	Mean width of spermatozoa head oscillation
BCF	Beat cross frequency	Hz	Frequency that spermatozoa track ¹ crosses spermatozoa path ²
VSL	Straight line velocity (or progressive velocity)	$\mu\text{m s}^{-1}$	Straight line distance from beginning to end of spermatozoa track divided by time elapsed
VCL	Curvilinear velocity (or track speed)	$\mu\text{m s}^{-1}$	Total distance between each measured position of spermatozoa track divided by time elapsed
VAP	Path velocity	$\mu\text{m s}^{-1}$	Total average path distance ³ for each spermatozoon divided by time elapsed
LIN	Linearity	%	Degree of departure from linear progression = VSL/VCL
STR	Straightness	%	VSL/VAP

¹The Hamilton-Thorn Motion Analyser calculates a cell centre of brightness for each frame (up to 30 frames s^{-1}), allowing a very close approximation to the position of the centre of mass of the sperm cell. Cells are tracked by comparing successive frames. A track is formed by joining the positions between succeeding frames with straight lines. This is therefore an electronic interpretation of the actual spermatozoan path.

²The actual path of the spermatozoa.

³A five-point running average position rate of change, giving a smoothed cell path velocity. It is the total distance along the average path for each sperm cell, divided by the time elapsed.

Spermatozoa used for insemination were prepared as described for the acrosome reaction experiment above, with 40% oviductal fluid–modified Tyrode’s medium (v/v) only, or with heparin. For this experiment, spermatozoa were incubated for 5 h in isthmic or ampullary oviductal fluid collected from three cows during nonluteal or luteal stages. Mature, cumulus-intact ova were washed three times in low bicarbonate-Hepes medium (Bavister, 1983) and 8–12 ova were transferred to each 50 μl fertilization medium microdrop. Spermatozoa previously incubated in oviductal fluid (2.5×10^5) were added and coincubated with ova for an additional 16–18 h at 39°C, 5% CO_2 . Penicillamine/hypotaurine/epinephrine solution was not added.

After coincubation, oocytes were removed from microdrops and washed. Cumulus was removed by passage through a 30 gauge needle. Oocytes were placed on a microscope slide with a minimal volume of low bicarbonate-Hepes medium. Oocytes were fixed in acid:alcohol (3:1 glacial acetic acid:95% (v/v) ethanol) for at least 24 h, and stained with 1% (w/v) aceto-orcein (Sirard *et al.*, 1988) just before evaluation under a microscope. Oocytes were considered fertilized if either two polar bodies were observed, or pronuclear chromatin was observed in a mitotic stage.

Analysis of total glycosaminoglycans

The colorimetric method of Kubajak and Ax (1985), based on that of Whiteman (1973) using Alcian blue dye, was used to determine total glycosaminoglycan concentration. Isthmic and ampullary oviductal fluids collected from four cows were analysed during nonluteal and luteal stages.

Statistical analyses

For each time point, the effect of region and oestrous cycle stage of oviductal fluid on motility, viability, glycosaminoglycan concentration, or each motion parameter (ALH, BCF, LIN, STR, VAP, VCL, VSL) was determined using general linear models analysis of variance (SAS, 1988). Fisher’s

protected least significant differences test was used to test the significance of bull, cow, region (ampulla or isthmus) and stage (nonluteal or luteal), as well as bull by cow and region by stage interactions. Comparisons were considered for region within stage and stage within region only. Significance with respect to pair-wise comparisons (region within stage or stage within region) were also noted for the motion analysis results.

The acrosome reaction data from three replicate experiments were standardized by expressing data as percentages of control values. For each time point in each experiment, the non-heparin-treated control spermatozoa (in modified Tyrode’s medium) were assessed for acrosome reaction, or for acrosome-reacted live and acrosome-reacted dead spermatozoa. Means of these control values were divided into each data point and multiplied by 100 to calculate a percentage of the control value. This value was then used to analyse the effect of region and oestrous cycle stage of oviductal fluid on acrosome-reacted, acrosome-reacted live or acrosome-reacted dead spermatozoa, using general linear models analysis of variance. Fisher’s protected least significant differences test was used to test the significance of region, stage and region by stage interactions. Comparisons were considered for region within stage and stage within region only.

General linear models analysis was also used to compare the effect of spermatozoa incubated in oviductal fluid from two regions and two oestrous cycle stages on fertilization success. Tukey’s Studentized range test was used to compare region within stage and stage within region only. Fertilization success of spermatozoa incubated with regional, stage-pooled oviductal fluid was compared with spermatozoa incubated with heparin by Student’s *t* test. Significance was accepted as $P \leq 0.05$ for all analyses.

Results

No differences in sperm viability, as analysed by eosin B–aniline blue stain, were found among oviductal fluid

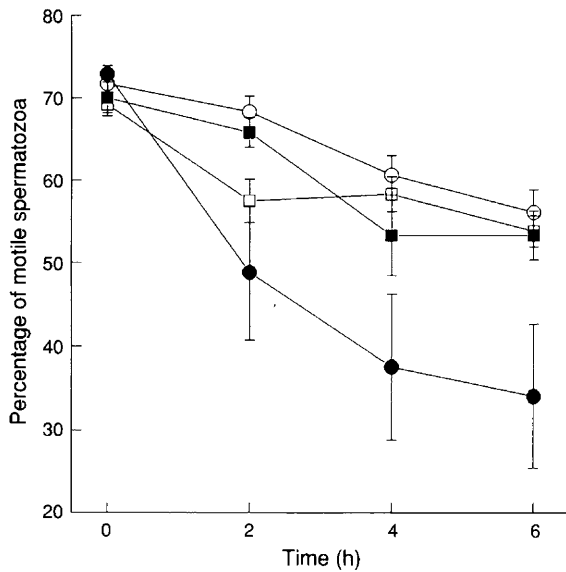


Fig. 1. Effect of oviductal fluid on sperm motility (mean \pm SEM). Spermatozoa were incubated for 0, 2, 4 or 6 h in 40% oviductal fluid collected from ampullary (squares) or isthmic (circles) regions from four cows during luteal (open) or nonluteal (closed) cycle stages. Sperm motility was assessed visually by a trained observer for the percentage of motile spermatozoa in each field; at least four fields per slide were measured. Significant differences were observed at: 2 h, ampullary nonluteal > isthmic nonluteal ($P = 0.046$), isthmic luteal > isthmic nonluteal ($P = 0.023$); 4 h isthmic luteal > isthmic nonluteal ($P = 0.029$); 6 h ampullary nonluteal > isthmic nonluteal ($P = 0.028$), isthmic luteal > isthmic nonluteal ($P = 0.010$).

treatments, or between oviductal fluid-treated and untreated control spermatozoa, over the 6 h incubation.

No differences were observed in sperm motility or acrosome reaction for incubation including 20% oviductal fluid. The percentage of motile spermatozoa did not differ with 40% oviductal fluid treatment at the first time point (Fig. 1). For the remainder of the 6 h incubation, isthmic nonluteal oviductal fluid suppressed motility significantly compared with incubation with other oviductal fluids.

Significant differences were observed in several motion parameters after incubation of spermatozoa with isthmic versus ampullary with 40% (v/v) oviductal fluid (Figs 2, 3). Regional effects were noted in lateral head movement and linearity in spermatozoa incubated for 2 h, and in straightness after 4 h incubation in oviductal fluid. Lateral head movement was greater ($P = 0.0342$) for spermatozoa incubated for 2 h in isthmic fluid compared with ampullary, while linearity was greater ($P = 0.0051$) after incubation for 2 h in ampullary fluid. Spermatozoa incubated in ampullary fluid for 4 h showed greater straightness than did spermatozoa incubated in isthmic fluid. Incubation for 2 h in luteal oviductal fluid resulted in greater average path velocity and curvilinear velocity compared with incubation in nonluteal oviductal fluid. When pair-wise comparisons are considered (stage within region or region within stage), spermatozoa incubated in isthmic oviductal fluid collected during the nonluteal stage demonstrated minimal lateral head movement, linearity, straightness, path velocity, curvilinear velocity and straight-line velocity compared with other types of fluids (Table 2).

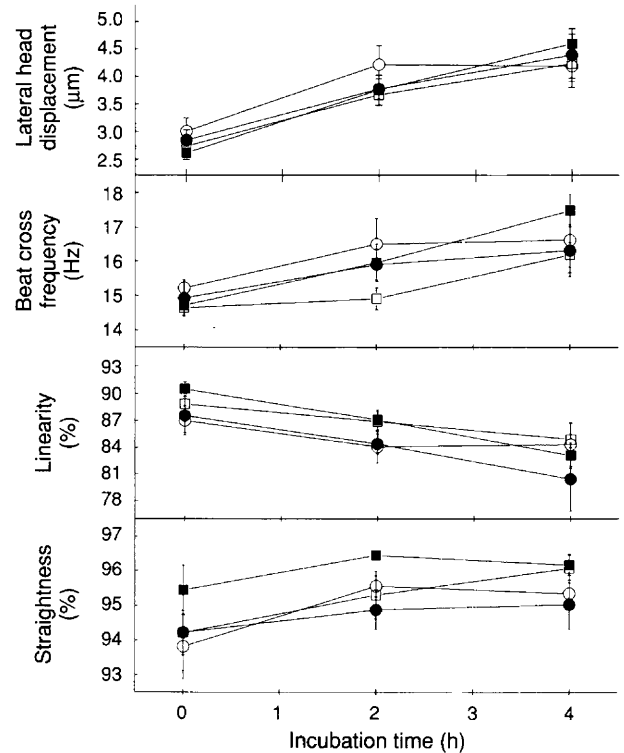


Fig. 2. Effect of oviductal fluid on four velocity parameters of sperm motility (mean \pm SEM). Spermatozoa were incubated for 0, 2 or 4 h in 40% oviductal fluid collected from ampullary (squares) or isthmic (circles) regions from three cows during luteal (open) or nonluteal (closed) cycle stages. Spermatozoa were assessed by the Hamilton-Thorn motion analyser for lateral head displacement, beat cross frequency, linearity and straightness. See Table 2 for list of significant differences observed.

Thus, incubation in isthmic nonluteal oviductal fluid caused a decrease in several types of sperm velocity, as well as linearity and straightness, compared with ampullary oviductal fluid.

Incubation of spermatozoa with 40% oviductal fluid assessed the ability of oviductal fluid to facilitate both sperm capacitation and the acrosome reaction. By 2 h, there was a significant increase ($P < 0.0024$) in the percentage of acrosome-reacted spermatozoa for isthmic nonluteal oviductal fluid (Fig. 4). This trend continued throughout incubation, so that at 6 h, spermatozoa incubated in isthmic nonluteal oviductal fluid acrosome reacted at a rate of 241% of control spermatozoa ($P = 0.0004$ compared with ampullary nonluteal and isthmic luteal incubations). Incubation in the other three oviductal fluid types caused sperm acrosome reactions at rates between 156 and 180% of control.

When acrosome-reacted live and acrosome-reacted dead spermatozoa were assessed, no statistical significance was noted between the oviductal fluid treatments (data not shown). Acrosome-reacted live spermatozoa ranged from 100–120% of control at 0 h to 200–250% of control at 4 h, and most acrosome-reacted live spermatozoa were found following incubation in ampullary nonluteal oviductal fluid. Acrosome-reacted dead spermatozoa were present at 95–115% of control at 0 h and 105–150% of control at 4 h. The maximum number

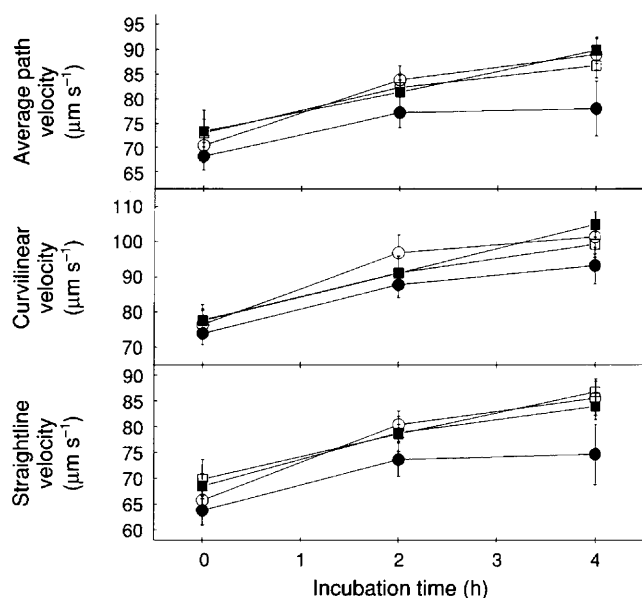


Fig. 3. Effect of oviductal fluid on three velocity parameters of sperm motility (mean \pm SEM). Spermatozoa were incubated for 0, 2 or 4 h in 40% oviductal fluid collected from ampullary (squares) or isthmic (circles) regions from three cows during luteal (open) or nonluteal (closed) cycle stages. Spermatozoa were assessed by the Hamilton–Thorn motion analyser for average path velocity, curvilinear velocity and straightline velocity. See Table 2 for list of significant differences observed.

of acrosome-reacted dead spermatozoa was found after incubation in isthmic nonluteal oviductal fluid for 4 h.

In vitro fertilization of bovine ova was highest (88.7%) after incubation of spermatozoa with 40% ampullary nonluteal oviductal fluid (Fig. 5). Both region and stage effects were noted. Incubation of spermatozoa with ampullary nonluteal oviductal fluid resulted in a significantly greater percentage of fertilized oocytes ($P < 0.05$) than did incubation in ampullary luteal or isthmic nonluteal oviductal fluid.

Mean total concentrations of glycosaminoglycan in oviductal fluid were 0.88 ± 0.05 mg ml⁻¹ for the ampulla and 0.77 ± 0.07 mg ml⁻¹ the isthmus ($n = 4$ cows). No significant differences in total glycosaminoglycans were detected among types of oviductal fluid (data not shown).

Discussion

Treatment with oviductal fluid did not affect sperm viability as assessed by staining techniques. The increase observed in the percentage of acrosome-reacted spermatozoa was not therefore a result of acrosome lysis following cell death. Viability did not differ between oviductal fluid-treated and nontreated controls, or between regions, or stages for oviductal fluid, or incubation times used for treatment.

Incubating bull spermatozoa in whole oviductal fluid (isthmic plus ampullary) increased linearity and vigorous motility, but decreased lateral head displacement and curvilinear velocity, compared with untreated spermatozoa (McNutt *et al.*, 1994). In the present study, we observed that the percentage of motile

Table 2. Statistically significant differences in sperm motility parameters after incubation in isthmic (I) or ampullary (A) oviductal fluid collected during luteal (L) or nonluteal (NL) oestrous cycle stages

Parameter	Incubation time	Incubation fluid	<i>P</i> value
Lateral head displacement	0 h	A L > A NL	0.028*
	2 h	I > A	0.034
		IL > A L	0.021
Beat cross frequency	2 h	IL > I NL	0.023*
		IL > A L	0.031
	Linearity	2 h	A > I
Straightness	2 h	A L > I L	0.024*
		A NL > I NL	0.049*
		A NL > A L	0.043*
		A NL > I NL	0.028*
Path velocity	2 h	A > I	0.051
		L > NL	0.043
	4 h	I L > I NL	0.021*
		A NL > I NL	0.033*
Curvilinear velocity	2 h	IL > I NL	0.021*
		L > NL	0.046
		IL > A L	0.038*
Straight line velocity	2 h	IL > I NL	0.015*
		IL > I NL	0.020*
	4 h	IL > I NL	0.023*
		A NL > I NL	0.030*

*Pairwise comparison only (stage within region *or* region within stage). See Figs 2 and 3 for graphical representation of data.

spermatozoa, as well as the three velocity parameters measured (path, curvilinear and straightline velocities), were lowest after incubation in isthmic nonluteal oviductal fluid versus ampullary fluid. These findings may be related to earlier observations that spermatozoa are quiescent in the isthmus before fertilization (Suarez *et al.*, 1990; reviewed by Ellington, 1991). Hunter and Wilmot (1984) proposed that spermatozoa may reside in a quiescent state in the bovine isthmus for up to 20 h before fertilization. Moreover, Cooper *et al.* (1979) noted reversible, depressed motility of spermatozoa in the lower oviduct of rabbits, and Suarez (1987) reported inhibition of flagellar movement in mouse oviducts, which may be a factor in detaining spermatozoa in the isthmus, and the creation of a sperm reservoir.

The greater velocities of spermatozoa incubated in ampullary oviductal fluid, as well as their increased linearity and straightness, suggest accelerated progressive sperm movement. Because spermatozoa exposed to ampullary oviductal fluid must move toward the ovum and generate force to penetrate the zona, their stimulated motility in this region is not unexpected.

Hyperactive motility of spermatozoa of many mammals may be associated with regulation of sperm transport in the oviduct (Suarez, 1987) or penetration of the zona pellucida (Fraser and Quinn, 1981; Fleming and Yanagimachi, 1982). Common accepted parameters of hyperactivation are decreased progressive movement (decreased linearity) and increased bending of the flagellum midpiece (increased lateral head movement)

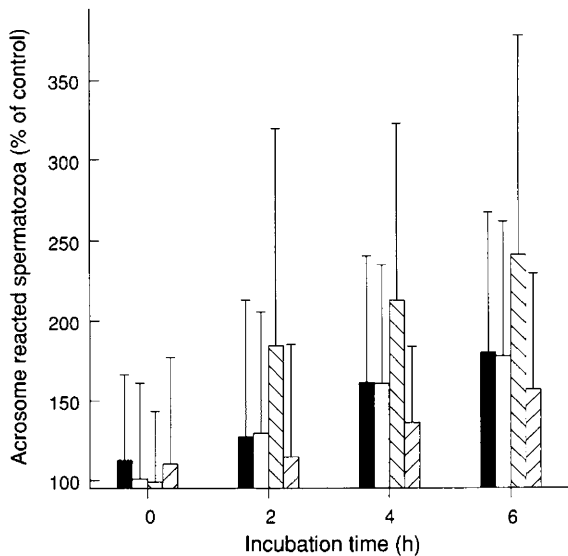


Fig. 4. Effect of oviductal fluid on the ability of spermatozoa to undergo the acrosome reaction (mean \pm SEM). Spermatozoa were incubated for 0, 2, 4 or 6 h in 40% oviductal fluid collected from ampullary or isthmic regions from 13 cows during luteal or nonluteal cycle stages (ampullary non-luteal, ■; ampullary luteal, □; isthmic nonluteal, ▨; isthmic luteal, ▩). Acrosome-reacted spermatozoa were assessed microscopically as the number of acrosome-reacted spermatozoa per 100 spermatozoa counted. This value was divided by the number of acrosome-reacted spermatozoa per 100 counted in control samples for the same incubation time. Significant differences were observed at: 2 h, isthmic nonluteal > ampullary nonluteal ($P = 0.0024$), isthmic nonluteal > isthmic luteal ($P = 0.0004$); 4 h, isthmic nonluteal > ampullary nonluteal ($P = 0.0004$), isthmic nonluteal > isthmic luteal ($P = 0.0004$); 6 h, isthmic nonluteal > ampullary nonluteal ($P = 0.0004$), isthmic nonluteal > isthmic luteal ($P = 0.0004$).

during vigorous movement. In terms of these parameters, our results do not support the notion of hyperactivation of bovine spermatozoa following incubation with ampullary oviductal fluid. However, no quantitative description of hyperactivation has been accepted for bovine spermatozoa, and McNutt *et al.* (1994) identified subpopulations of bovine spermatozoa that exhibit more classic hyperactivation characteristics. McNutt *et al.* (1994) analysed bovine spermatozoa incubated with whole oviductal fluid for 0–4 h by computer-assisted automation identical to that used in the present study. Three-dimensional plots of curvilinear velocity versus lateral head displacement versus frequency showed that ‘hyperactive’ spermatozoa were found to constitute a smaller percentage (16%) of the total spermatozoa population, compared with untreated control spermatozoa. A similar subpopulation of hyperactivated spermatozoa may have been present among those analysed in this study, although this was not examined.

In the present study, the number of acrosome reactions was high for spermatozoa incubated in isthmic nonluteal oviductal fluid, even at 2 h. The absence of differences in osmolality and pH among the 40% oviductal fractions precludes nonspecific acrosome reactions as a cause of this. These data therefore support the concept of the oviduct isthmus as a sperm reservoir, which provides a population of capacitated, acrosome-reacted spermatozoa at the time of ovulation. Smith

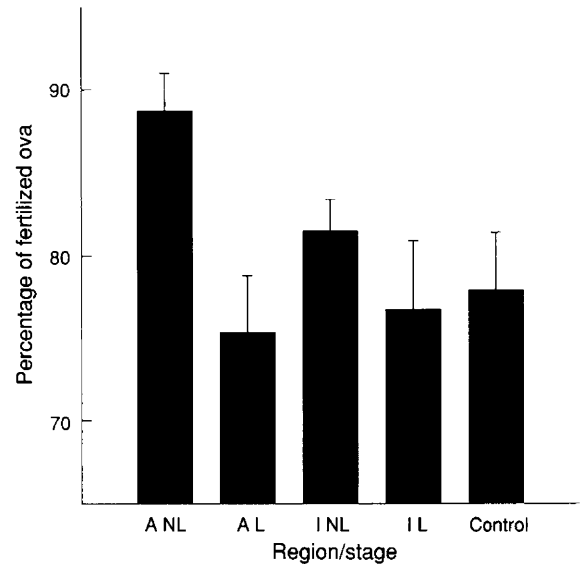


Fig. 5. Effect of oviductal fluid on sperm fertilizing ability (mean \pm SEM). Spermatozoa were incubated for 5 h in 40% oviductal fluid collected from ampullary (A) or isthmic (I) regions from three cows during luteal (L) or nonluteal (NL) cycle stages. Fertilizing ability was assessed as the percentage of ova fertilized after 16–18 h incubation with spermatozoa. Significant differences were observed as follows: ampullary nonluteal > isthmic nonluteal ($P = 0.041$), ampullary nonluteal > ampullary luteal ($P = 0.009$).

and Yanagimachi (1989) reported that the hamster oviduct apparently controls the timing of sperm capacitation to synchronize the state of the spermatozoa with ovulation, thereby maximizing the chances of fertilization. The isthmic environment may serve to facilitate capacitation and the acrosome reaction to prepare a reservoir, or population of spermatozoa, available for gradual release to fertilize the ovum. As such, the isthmus may limit the number of functionally competent spermatozoa, thus reducing the possibility of polyspermy.

The greatest ovum fertilization rate occurred using spermatozoa incubated with ampullary nonluteal fluid. These results suggest that ampullary oviductal fluid may further prepare spermatozoa released from the isthmus to achieve maximal fertilization rates at the site at the time when the ovum is present.

Cyclic variations in the composition of bovine regional oviductal fluid are evident. A combination of factors in the oviduct probably optimizes the preparation of spermatozoa for fertilization. Higher calcium concentrations were found in isthmic nonluteal oviductal fluid compared with isthmic luteal or ampullary nonluteal oviductal fluid (Grippo *et al.*, 1992). High calcium concentrations may increase the occurrence of the acrosome reaction in spermatozoa incubated in isthmic nonluteal oviductal fluid. Increased concentrations of lysophosphatidylcholine were also found in ampullary oviductal fluid collected during the nonluteal stage (Grippo *et al.*, 1994). This fusogenic lysophospholipid may service to maximize the number of acrosome-reacted spermatozoa in the ampulla at fertilization. Oestrus-associated protein is present in oviductal fluid primarily during the nonluteal stage (Gerena and Killian, 1990) and in a higher concentration in ampullary-derived fluid than in isthmic fluid (Wegner and Killian, 1992).

Oestrus-associated protein is associated with the sperm membrane, and stimulates increased capacitation and fertilization of bovine spermatozoa (King *et al.*, 1994).

Proteins and proteoglycans produced by the isthmus at oestrus are believed to play a major role in the abilities of conditioned medium from bovine explants to capacitate spermatozoa (Anderson and Killian, 1994). These authors also reported higher concentrations of glycosaminoglycans in isthmic/oestrus conditioned medium, compared with ampullary/oestrus or isthmic/luteal media. In the present study, differences between concentrations of glycosaminoglycans in oviductal fluid from the two regions or the two stages were not observed, suggesting that they are not solely responsible for observed differences in sperm acrosome reaction, as proposed by Parrish *et al.* (1989). The disparity in concentrations of glycosaminoglycans noted in oviductal fluid compared with that of the conditioned medium may be due to sample handling. In the conditioned medium preparations, molecules of <10 kDa (including free glycosaminoglycans) were excluded, and all preparations were standardized against protein concentration. Both free glycosaminoglycans and proteoglycans were measured, as well as smaller biological molecules that may affect sperm motility, capacitation and the acrosome reaction. It is likely that many biologically active molecules and biochemical reactions could account for the observed increased fertilization of bovine ova by bovine spermatozoa in the ampullary environment at the time of ovulation.

The results of this study separate oviductal regional and oestrous cycle stage effects on sperm physiology. We demonstrated that oviductal fluid collected from the isthmus during the nonluteal stage, although lowering sperm motility, optimizes the ability of spermatozoa to undergo the acrosome reaction, without diminishing their viability. These effects may serve to increase the number of spermatozoa prepared to fertilize the ovum in the ampulla during the nonluteal stage.

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