Journal of Andrology, Vol. 13, No. 1, January/February 1992
Copyright © American Society of Andrology

Movement Characteristics of Boar Sperm Obtained from the Oviduct or Hyperactivated *In Vitro*

SUSAN S. SUAREZ, XIAO-BING DAI, ROBERT P. DEMOTT, KEMBER REDFERN, AND MARK A. MIRANDO*

*From the Department of Physiological Sciences, College of Veterinary Medicine, and the *Department of Animal Science, University of Florida, Gainesville, Florida.*

ABSTRACT: The objectives of this study were to describe hyperactivated motility in boar sperm and to determine the incidence of hyperactivation among boar sperm flushed from the oviduct. Oviducts were surgically removed from 13 gilts 32 hours after mating them to fertile boars. The majority of the sperm flushed from the oviducts was immotile, weakly motile, or stuck to mucus or cellular debris. The mucus could not be penetrated by the sperm. The remaining 3% to 19% of the flushed sperm was free-swimming. Only five hyperactivated sperm were recovered, all from the ampulla of the oviduct. The remainder of the free-swimming sperm travelled in linear trajectories and possessed significantly higher flagellar curvature ratios (the flagella were less bent) than boar sperm measured in diluted semen. Hyperactivated motility was induced in washed ejaculated boar sperm, using a 1-minute pulse of 4 μmol/L calcium ionophore A23187. The ionophore-treated sperm had significantly lower straight-line velocities, linearities, and flagellar curvature ratios than controls, as would be expected for hyperactivated sperm. They were vigorous and swam in circles. It was concluded that, although few hyperactivated boar sperm could be recovered from the oviduct, boar sperm are capable of undergoing hyperactivation.

Key words: Pig, uterine tube, fallopian tube, sperm motility.
J Androl 1992;13:75–80.

In several species, sperm flushed from the ampulla of the oviduct have exhibited hyperactivated motility (guinea pig: Yanagimachi and Mahi, 1976; rabbit: Cooper et al, 1979, Suarez et al, 1983; sheep: Cummins, 1982; mouse: Olds-Clarke, 1986; hamster: Smith and Yanagimachi, 1989; Smith and Yanagimachi, 1990). This movement pattern is characterized by highly asymmetrical flagellar bending and less progressive movement on glass microscope slides than sperm taken from an ejaculate or the caudal epididymis. In this study, following a suggestion by Yanagimachi (1981), the highly progressive movement pattern displayed by most sperm in the ejaculate or recovered from the caudal epididymis will be referred to as "activated," to distinguish these sperm from hyperactivated sperm. Several functions have been proposed for hyperactivation. It may aid the sperm in penetrating oviductal mucus or the matrix of the cumulus oophorus. Hyperactivated hamster sperm have been found to be more capable of penetrating viscous media than activated hamster sperm (Suarez et al, 1991a). Hyperactivation may also help sperm escape from pockets or troughs created by mucosal folds in the oviduct (Suarez, 1987; Smith and Yanagimachi, 1989; Smith and Yanagimachi, 1990). Finally, hyperactivation may assist in the penetration of the zona pellucida (Suarez et al, 1984; Katz et al, 1989).

There have been anecdotal reports of hyperactivation occurring in boar sperm during capacitation *in vitro* (Saxena et al, 1986; Hamano et al, 1989). Nevertheless, the movement pattern has not been illustrated or described. To our knowledge, hyperactivated boar sperm have not been recovered from the porcine oviduct. The following experiments were undertaken to describe hyperactivated motility in boar sperm, and to determine the incidence of hyperactivated motility among sperm flushed from the oviduct of the pig.

Materials and Methods

Chemicals and Media

Chemicals were obtained from Sigma Chemical Company (St. Louis, MO) unless otherwise stated. Oviduct flushing medium (OFM) consisted of Earle's salts supplemented with 2.92 mmol/L calcium lactate, 0.91 mmol/L sodium pyruvate, 0.5 mmol/L hypotaurine, 0.0075% penicillin G; 0.005% streptomycin, 24.9 mmol/L sodium bicarbonate, 10 mmol/L N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; Calbiochem, La Jolla, CA), and 5 mg/ml porcine serum albumin. This medium was based on the boar sperm capacitation medium used by Saxena et al (1986), with hypotaurine added to support sperm motility (Meizel et al, 1980). For the second set of experiments, calcium lactate was replaced by sodium lactate. The pH was adjusted to

Supported by USDA grant 88-37242-4910 and NIH grant HD-19854. Florida Agricultural Experiment Station journal series number: R-01581.

Correspondence to: Susan S. Suarez, PhD, Department of Physiological Sciences, College of Veterinary Medicine, Box J-144 Health Science Center, University of Florida, Gainesville, Florida 32610-0144.

Received for publication May 29, 1991; accepted for publication August 9, 1991.

reach 7.6 in air after filter sterilizing. Hyperactivating medium (HM) consisted of 110 mmol/L NaCl, 5 mmol/L KCl, 0.36 mmol/L NaH_2PO_4, 24.9 mmol/L $NaHCO_3$, 0.49 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 25 mmol/L HEPES, 5 mmol/L glucose, 0.125 mmol/L sodium pyruvate, 6.26 mmol/L sodium lactate, 0.5 mmol/L hypotaurine, 0.006% Na penicillin-G, and 1 mg/ml Fraction V bovine serum albumin (BSA; Calbiochem), pH 7.6. This medium was modified from a hamster sperm hyperactivating medium (Suarez, 1988) by lowering the calcium to increase survival after ionophore addition, and by lowering the BSA initially so more could be added to absorb ionophore after the pulse treatment described below.

Sperm Recovery from Oviducts

Sexually mature, crossbred gilts (Duroc × Yorkshire × Hampshire) were checked daily for behavioral estrus at 7:30 AM using fertile boars. Ten gilts were mated to boars of proven fertility at 8:00 AM on the morning in which lordosis was first observed. Oviducts were obtained 32 hours after mating. Based on our experience, this was the expected time for the beginning of ovulation.

To obtain oviducts, general anesthesia was induced using 1 g thiamylal sodium (intravenous), and was maintained with 3% to 5% halothane. A ventral midline incision was made aseptically, and both oviducts were exposed. Small hemostats were placed in the following order to isolate segments of the oviducts: 1) 2 cm above the uterotubal junction, 2) at the uterotubal junction, 3) at the ampullary–isthmic junction, and 4) at the fimbria. Oviducts were excised, placed in enough 37°C Earle's salts solution to cover, and transported in an insulated container to the laboratory. There, each segment was separated and flushed with 50 μl OFM into a 35-mm Petri dish containing 3 ml of 37°C silicon oil that had been equilibrated with 5% carbon dioxide in humidified air. The dish was kept in an incubator set at 37°C and 5% carbon dioxide until the sperm were videotaped.

In another set of experiments, three gilts in estrus were mated twice, 12 hours apart, and taken to surgery 32 hours after the first mating. Oviducts were exposed as described above, but were not divided into segments. They were immediately flushed with medium while still in the surgical field. Flushings were kept at 37°C and under silicon oil during transport to the laboratory.

In order to observe and videotape sperm movement, 30 μl of flushing was placed on an agar-coated slide (Suarez et al, 1991a) and surrounded with equilibrated silicon oil. The coverslip was supported on two edges by single layers of Parafilm (American Can Company, Greenwich, CT).

Hyperactivation In Vitro

Boar semen was collected through Miracloth (Calbiochem, La Jolla, CA) into a thermos using the "gloved hand" technique (Hancock and Hovell, 1959). Three boars of proven fertility were used for these experiments. Sperm were washed within 30 minutes of collection, as described previously (Suarez et al, 1991b). In brief, semen was diluted in a 1:1 ratio with HM (final volume 15 ml) and centrifuged twice for 10 minutes at 15 × g to remove debris. Sperm were washed twice by diluting to 15 ml with HM and centrifuging for 5 minutes at 170 × g. Sperm collected in the final pellet were diluted to a concentration of 2×10^7/ml. Motility of the washed sperm exceeded 80%.

Hyperactivation was induced by exposing the sperm to a pulse of calcium ionophore A23187 (Suarez et al, 1987) as follows: 20 μl of 100 μmol/L A23187 in dimethylsulfoxide (DMSO) was mixed with 80 μl of HM, then added to 400 μl of sperm suspension. This was incubated in the dark at 37°C for 1 minute, 500 μl of HM containing 40 mg/ml BSA was added, and sperm were incubated for 2 minutes. Samples were placed in slide chambers as described above and videotaped. Controls were treated in a similar fashion, using DMSO alone.

Videomicroscopy and Image Analysis

Slides were placed on a Zeiss Axiovert (Carl Zeiss Inc., Thornwood, NY) microscope stage heated to 37°C, and sperm were videotaped through a ×40 differential interference contrast objective using a black-and-white CCD camera (CCD 72, Dage MTI, Michigan City, IN). The light source was a xenon stroboscope (Chadwick Helmuth Model 10030, El Monte, CA) that flashed at a rate of 60 Hz. Light passed through a red filter to minimize damage to sperm. For some experiments, a microchannel plate image intensifier (Gen II Sys, Dage MTI) with bandwidth control was used to create a stroboscopic effect. The video image and a signal of the passage of time in 0.01-second intervals (ForA video time/date generator VTG33, Los Angeles, CA) were recorded on a Panasonic AG 7300 Super VHS video cassette recorder.

For the sperm flushed from oviducts, the first 50 appearing in the videotapes (if that many were recovered) were categorized as hyperactivated, activated, stuck, vibratory, or immotile. Sperm were characterized as hyperactivated if they swam in the patterns defined as hyperactivated for rabbit sperm (Suarez et al, 1983), which are of similar shape and size to boar sperm. The term "activated" refers to free-swimming sperm actively swimming in nearly straight trajectories. Sperm that were active but lodged in mucus or cells were characterized as being stuck. Sperm whose flagella were beating weakly were categorized as vibratory.

The following movement variables were measured on sperm that were categorized as hyperactivated or activated, as well as those treated to hyperactivation *in vitro*: straight-line velocity (VSL), curvilinear velocity (VCL30), linearity (VSL/VCL30; Olds-Clarke, 1989), and flagellar curvature ratio (FCR; Suarez, 1988). The FCR (Fig 1) is an indicator of flagellar bending that, in our experience, offers greater precision than flagellar bend amplitude and wavelength. Measurements were taken on the first sperm to enter the center of each video field. Velocity measurements were taken over a period of 1 second, while FCR was taken from the video frame in which the flagellar bend had reached its peak. Measurements were made directly on a Panasonic model TR-196M black-and-white video monitor using a Grafbar GP7 Sonic Digitizer (Science Accessories Corp., Stratford, CT). Signals generated by the digitizer passed into a MacIntosh 512k computer (Apple Computers, Inc., Cupertino, CA), where they were converted to measurements using a program in BASIC written by W. Gottlieb and R. P. DeMott. Data were analyzed by analysis of variance. Differences with a probability of chance occurrence below 0.05 were considered to be significant.

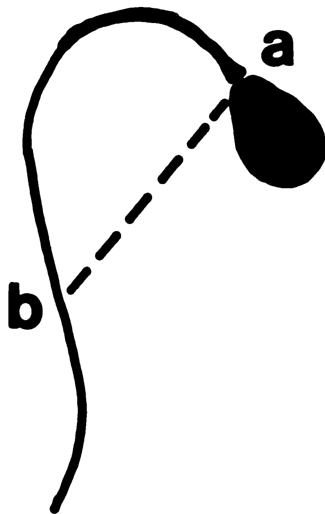

FIG. 1. Measurement of flagellar curvature ratio (FCR). The ratio is the straight-line distance from **a** (the head/middle piece junction) to **b** (the first inflection point along the tail), divided by the curvilinear distance from **a** to **b** along the tail.

Scanning Electron Microscopy

A gilt was mated twice as described above, and oviducts were recovered surgically. They were immediately pinned to a wax dissecting platform, slit open longitudinally with a #11 scalpel, and pinned open. Fixative was dripped over the preparation, it was covered with plastic wrap to prevent evaporation, and the tissue was allowed to fix for 30 minutes. Fixative contained 2% glutaraldehyde, 2% formaldehyde, and 0.01% $CaCl_2$ in 0.01 mol/L phosphate buffer, pH 7.3, with 0.5% cetyl pyridinium chloride added to preserve mucus (Pearse, 1985). Segments of the isthmus were removed and transferred to vials of fixative. Tissue was dehydrated, processed through critical point-drying, and sputter-coated with gold for scanning electron microscopy. Tissue was scanned and photographed on a Hitachi (Rolling Meadows, IL) S4000 Scanning Electron Microscope at the Interdisciplinary Center for Biotechnology Research, Electron Microscopy Core Laboratory of the University of Florida.

Results

Ten gilts were used in the first experiment. The ovaries observed during surgery were found to be preovulatory in five gilts, periovulatory in three gilts, and postovulatory in two gilts. Only three sperm (two from an ovulating gilt and one from a preovulatory gilt) were recovered that exhibited obvious hyperactivated motility; all were from the ampulla. The isthmic segments were particularly difficult to flush. This may have been due to the small size of the lumen and to the presence of a granular-appearing mucous substance (Fig 2) that was seen on most of the slides. Sperm were trapped by this mucus and were not observed to free themselves. Most of the sperm were recovered from the isthmus. In only two gilts were more than three sperm recovered from an ampulla. In these cases (periovulatory and postovulatory), less than 50 sperm were recovered, and all but a few were immotile. Of sperm recovered from both the ce-

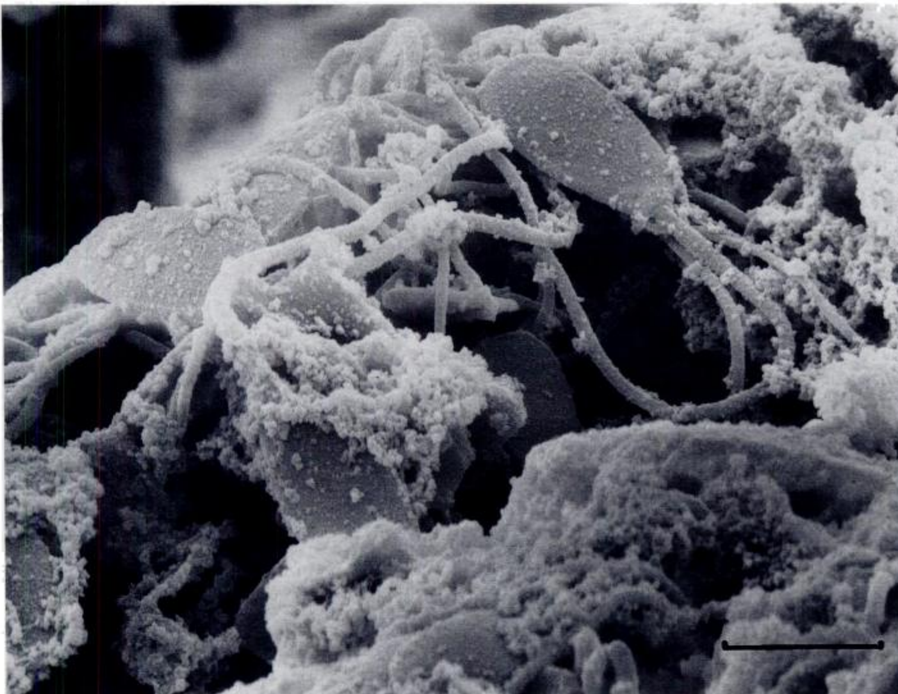

FIG. 2. Scanning electron micrograph of boar sperm trapped in a mucous substance in the isthmus of the porcine oviduct (bar = 5 μm).

phalic and caudal segments of the isthmus, the median percentages in each category (ranges given in parentheses) were: 4% activated (3% to 19%), 15% stuck (7% to 20%), 8% vibratory (2% to 12%), and 78% immotile (52% to 94%).

In an attempt to increase sperm recovery, three gilts were mated twice to two different boars, and the oviducts were flushed with about 5 ml medium at the site of surgery, rather than in the laboratory. Ovulation had occurred by the time of surgery in all three gilts. Sperm were then concentrated by centrifugation at 450 × g for 5 minutes. The median percentage of activated sperm was 8% (range, 6% to 12%), 66% stuck (63% to 72%), and 25% immotile (20% to 28%). Only two sperm were hyperactivated, both from the same gilt.

Movement variables were measured for sperm recovered from cephalic and caudal isthmic segments of three periovulatory gilts and from medium-extended semen samples from three boars. Variability of velocity data was high, and significant effects were detected for experiment, side, and segment of the tract; however, variability for flagellar curvature ratio (FCR) was low, and no effect of experiment, side, or segment could be detected ($P > 0.7$ by ANOVA). Surprisingly, the oviductal sperm had significantly higher FCRs ($P < 0.0001$) than sperm measured in semen (Table 1), indicating that the flagellar bending had decreased. We had to use a different boar for each experiment, so we performed an analysis of variance test on FCR values pooled by experiment. Variation in FCR values between experiments was much less than variation between treatment groups (Table 2).

Since only five free-swimming hyperactivated sperm were found among the flushes, an attempt was made to determine whether a higher proportion of boar sperm could hyperactivate *in vitro*. This was done by treating sperm obtained from three different fertile boars with a 4 μmol/L pulse of calcium ionophore A23187. The movement pattern of about 30% of the ionophore-treated sperm matched that of hyperactivated rabbit sperm flushed from the oviduct (Suarez et al, 1983), and the measurements are given in Table 3. It appeared from single frames of the videotape that the acrosomes were intact in both the control and ionophore-treated sperm. The FCR, VSL, and VSL/VCL30 were significantly reduced among these sperm, compared to controls, as would be expected for hyperactivated sperm. In the third ionophore experiment, the effect of the ionophore was greater than in the other two, so a significant interaction

Table 1. *Mean flagellar curvature ratios (FCR × 100) of boar sperm flushed from all isthmic segments of periovulatory oviducts compared with those of boar sperm in seminal plasma**

	Flagellar curvature ratio		Number of sperm
	Mean ± SD	Range	
Sperm in semen	81.8 ± 9.1	51.1–91.1	20
	79.1 ± 8.8	62.4–89.8	20
	88.2 ± 6.0	68.5–96.1	20
Overall mean	82.8 ± 8.8		
Oviductal sperm	94.9 ± 6.0	81.8–98.9	7
	95.3 ± 3.8	84.0–99.2	47
	96.7 ± 1.8	93.9–98.8	5
Overall mean	95.4 ± 3.9		

* Plasma was diluted 1:10 with oviduct flushing medium. Lower numbers represent more sharply curved flagellar bends. The FCRs of oviductal sperm were significantly higher ($P < 0.0001$) than those of sperm in seminal plasma.

Table 2. *One-factor, nonrepeated measured analysis of variance table for FCR values pooled by experiment**

Source	DF	Sum sq.	Mean sq.	F-test
Between groups	1	0.024	0.024	20.948
Within groups	4	0.005	0.001	
Total	5	0.028		P = 0.010

* Treatment groups = sperm in diluted semen and oviductal sperm.

Table 3. *Movement variables measured for boar sperm induced to hyperactivate* in vitro*

	Ionophore-treated	Control
VSL (μm/second)†	11.2 ± 6.22	59.2 ± 14.52
	9.0 ± 6.73	59.1 ± 10.11
	10.9 ± 6.42	58.0 ± 13.06
Mean	10.4 ± 6.45	58.8 ± 12.56
VCL30 (μm/second)	67.8 ± 9.15	67.8 ± 14.80
	74.4 ± 11.25	68.6 ± 9.87
	85.2 ± 24.49	75.6 ± 17.17
Mean	75.8 ± 14.96	70.7 ± 13.95
VSL/VCL30 (×100)†	16.8 ± 9.4	86.8 ± 6.0
	12.3 ± 8.8	86.1 ± 5.7
	13.0 ± 7.5	77.1 ± 9.0
Mean	14.0 ± 8.6	83.3 ± 6.9
FCR (×100)‡	57.4 ± 11.0	92.7 ± 3.7
	54.6 ± 13.2	94.2 ± 2.6
	33.8 ± 8.1	92.8 ± 4.7
Mean	48.6 ± 10.8	93.2 ± 3.7

* Means (±SD) are reported for 20 sperm per treatment. Sperm were collected from three different boars of proven fertility. In brief, VSL is the net distance travelled by the head/midpiece junction in 1 second, and VCL30 is the total distance travelled by the head/midpiece junction in 1 second, with the position of the junction being taken at 1/30-second intervals (Olds-Clarke, 1989).

† Effect of treatment was significant ($P < 0.0001$).

‡ Effect of treatment was significant ($P < 0.0001$), and a significant interaction was detected between treatment and boar ($P < 0.0001$).

was detected between boar and treatment in the analysis of variance. Tracings of these sperm appear in Figure 3.

Discussion

We found only a few hyperactivated sperm in our flushings of gilt oviducts. It might be argued that our methods or medium were inadequate to support hyperactivation; however, we did include components known to be required by other species (hypotaurine: Meizel et al, 1980; glucose, lactate, and pyruvate: Dravland and Meizel, 1981; Fraser and Quinn, 1981; albumin or polyvinyl alcohol: Bavister, 1981; bicarbonate and carbon dioxide: Neill and Olds-Clarke, 1987; Boatman and Robbins, 1991). Others have also recovered only low numbers of hyperactivated sperm from oviducts of naturally mated animals. In rabbits, about 10 sperm could be recovered from both ampullae of each periovulatory doe, and about 9 of these were hyperactivated (Suarez et al, 1983). In the ewe, less than 50% of the 10 to 100 sperm flushed from each ampulla were hyperactivated (Cummins, 1982). When sperm were observed directly through the ampullar walls of oviducts excised from hamsters (Katz and Yanagimachi, 1980) and mice (Suarez, 1987), only a few free-swimming hyperactivated sperm were ever seen at any time. Some of these numbers of hyperactivated sperm are higher than the numbers we found, but they still amounted to only a few per animal. Thus, it is reasonable to propose that we recovered only a few hyperactivated sperm from the ampulla because there were few present.

Higher numbers of sperm were recovered from the isthmus than the ampulla, but many of them were immotile or trapped in mucus. These results are similar to those of Smith and Yanagimachi (1990), who found many immotile sperm amidst mucus and cellular debris when they flushed the hamster isthmus. Their evidence indicates that most of the motile sperm were trapped between folds of mucosa. One of us (Suarez, 1987) has found a similar distribution of motile and immotile mouse sperm *in vivo* by using transillumination to look into the oviduct. Using scanning electron microscopy to examine the porcine oviduct, Hunter and Flechon (Flechon and Hunter, 1981; Hunter et al, 1987) found sperm attached to the epithelial surface deep within the folds of the caudal isthmus. Thus, we may have been unable to recover all of the motile boar sperm present in the isthmus because some were sequestered in mucosal pockets and/or bound to the epithelium. Such sperm may or may not have been hyperactivated.

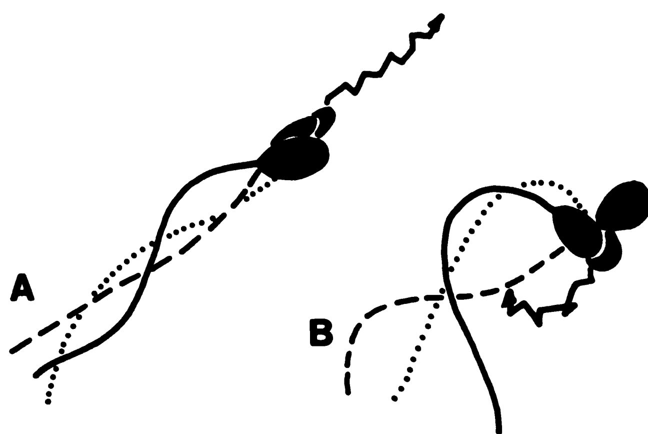

FIG. 3. Patterns of activated (A) and hyperactivated (B) movement. These figures were traced from sequential frames of videotape, at a framing rate of 30 Hz. The kinked solid line represents the path taken by the head/middle piece junction.

The granular mucous material that was recovered from the isthmus and was apparent in our scanning electron micrographs was probably similar to material observed in scanning electron micrographs of oviductal isthmuses from pigs (Flechon and Hunter, 1981; Hunter et al, 1987), rabbits (Jansen, 1978; Jansen and Bajpai, 1982), and humans (Jansen, 1980). This material also appeared in explant cultures of isthmic mucosa taken from gilts (Suarez et al, 1991b) and cows (Suarez et al, 1990). Almost all of the sperm that were trapped in this material failed to release themselves, providing additional evidence that oviductal mucus could play a role in the formation of an isthmic reservoir by interfering with sperm ascent to the ampulla.

Although few hyperactivated boar sperm could be recovered from the oviduct, a large proportion responded *in vitro* to treatment with calcium ionophore A23187. These sperm displayed a similar pattern to that of rabbit (Suarez et al, 1983), ram (Cummins, 1982), and bull (Suarez and Pollard, unpublished data) sperm, which are all of similar size and shape. It was surprising that FCR values were higher among oviductal sperm than sperm in semen, indicating either that sperm had straightened at some point in the female tract or that some selection of sperm had taken place in the female. It is possible that the removal of seminal plasma was responsible for the increase in FCR values, since the control sperm in the ionophore experiments had been washed free of seminal plasma, and had FCR values similar to the oviductal sperm.

In summary, very few hyperactivated sperm were recovered from the porcine oviduct, but hyperactivation could be induced *in vitro* with calcium ionophore. Many of the sperm that could be flushed from the isthmus were immotile and/or trapped in a granular mucous substance. In the pig, only a few sperm may reach the ampulla and hyperactivate.

Acknowledgments

The authors thank Drs. Samir S. Raychoudhury and Gregory W. Erdos for technical assistance with scanning electron microscopy, and Dr. Fuller

Bazer and members of his laboratory for assistance with estrus detection and surgery.

References

Bavister BD. Substitution of a synthetic polymer for protein in a mammalian gamete culture system. *J Exp Zool.* 1981;217:45–51.

Boatman DE, Robbins RS. Bicarbonate: carbon-dioxide regulation of sperm capacitation, hyperactivated motility, and acrosome reactions. *Biol Reprod.* 1991;44:806–813.

Cooper GW, Overstreet JW, Katz DF. The motility of rabbit spermatozoa recovered from the female reproductive tract. *Gamete Res.* 1979;2:35–42.

Cummins JM. Hyperactivated motility patterns of ram spermatozoa recovered from oviducts of mated ewes. *Gamete Res.* 1982;6:53–64.

Dravland E, Meizel S. Stimulation of hamster sperm capacitation and acrosome reaction in vitro by glucose and lactate and inhibition by the glycolytic inhibitor, α-chlorohydrin. *Gamete Res.* 1981;4:515–523.

Flechon J-E, Hunter RHF. Distribution of spermatozoa in the utero-tubal junction and isthmus of pigs, and their relationship with the luminal epithelium after mating: a scanning electron microscope study. *Tissue Cell.* 1981;13:127–139.

Fraser LR, Quinn PJ. A glycolytic product is obligatory for initiation of the sperm acrosome reaction and whiplash motility required for fertilization in the mouse. *J Reprod Fertil.* 1981;61:25–35.

Hamano S, Naito K, Fukada Y, Toyoda Y. In vitro capacitation of boar ejaculated spermatozoa: effect of conditioned media prepared from preincubated sperm suspension. *Gamete Res.* 1989;24:483–489.

Hancock JL, Hovell GJR. The collection of boar semen. *Vet Rec.* 1959; 71:664–665.

Hunter RHF, Flechon B, Flechon J-E. Pre- and peri-ovulatory distribution of viable spermatozoa in the pig oviduct: a scanning electron microscope study. *Tissue Cell.* 1987;19:423–436.

Jansen RPS. Fallopian tube isthmic mucus and ovum transport. *Science.* 1978;201:349–351.

Jansen RPS. Cyclical changes in the human fallopian tube isthmus and their functional importance. *Am J Obstet Gynecol.* 1980;136:292–308.

Jansen RPS, Bajpai VK. Oviduct acid mucus glycoproteins in the estrous rabbit: ultrastructure and histochemistry. *Biol Reprod.* 1982;26:155–168.

Katz DF, Yanagimachi R. Movement characteristics of hamster spermatozoa within the oviduct. *Biol Reprod.* 1980;22:759–764.

Katz DF, Overstreet JW, Drobnis EZ. Factors regulating mammalian sperm migration through the female reproductive tract and oocyte vestments. *Gamete Res.* 1989;22:443–469.

Meizel S, Lui CW, Working PK, Mrsny RJ. Taurine and hypotaurine: their effects on motility, capacitation and the acrosome reaction of hamster sperm in vitro and their presence in sperm and reproductive tract fluids of several mammals. *Development, Growth and Differentiation.* 1980;22:483–494.

Neill JM, Olds-Clarke P. A computer-assisted assay for mouse sperm hyperactivation demonstrates that bicarbonate but not bovine serum albumin is required. *Gamete Res.* 1987;18:121–140.

Olds-Clarke P. Motility characteristics of sperm from the uterus and oviducts of female mice after mating to congenic males differing in sperm transport and fertility. *Biol Reprod.* 1986;34:453–467.

Olds-Clarke P. Sperm from $t^{w32}/+$ mice: capacitation is normal, but hyperactivation is premature and nonhyperactivated sperm are slow. *Dev Biol.* 1989;131:475–482.

Pearse AGE. *Histochemistry: Theoretical and Applied, Vol 2: Analytical Technology.* New York: Churchill Livingston; 1985.

Saxena N, Peterson RN, Sharif S, Saxena NK, Russell LD. Changes in the organization of surface antigens during in-vitro capacitation of boar spermatozoa as detected by monoclonal antibodies. *Reprod Fertil.* 1986;78:601–614.

Smith TT, Yanagimachi R. Capacitation status of hamster spermatozoa in the oviduct at various times after mating. *J Reprod Fertil.* 1989;86: 255–261.

Smith TT, Yanagimachi R. The viability of hamster spermatozoa stored in the isthmus of the oviduct: the importance of sperm–epithelium contact for sperm survival. *Biol Reprod.* 1990;42:450–457.

Suarez SS. Sperm transport and motility in the mouse oviduct: observations in situ. *Biol Reprod.* 1987;36:203–210.

Suarez S. Hamster sperm motility transformation during development of hyperactivation in vitro and epididymal maturation. *Gamete Res.* 1988; 19:51–65.

Suarez SS, Katz DF, Overstreet JW. Movement characteristics and acrosomal status of rabbit spermatozoa recovered at the site and time of fertilization. *Biol Reprod.* 1983; 29:1277–1287.

Suarez SS, Katz DF, Meizel S. Changes in motility that accompany the acrosome reaction in hyperactivated hamster spermatozoa. *Gamete Res.* 1984; 10:253–265.

Suarez SS, Vincenti L, Ceglia MW. Hyperactivated motility induced in mouse sperm by calcium ionophore A23187 is reversible. *J Exp Zool.* 1987;244:331–336.

Suarez SS, Drost M, Redfern K, Gottlieb W. Sperm motility in the oviduct. In: Bavister BD, Cummins J, Roldan ERS, eds. *Fertilization in Mammals.* Norwell, MA: Serono Symposia, USA; 1990:111–124.

Suarez SS, Katz DF, Owen DH, Andrew JB, Powell RL. Evidence for the function of hyperactivated motility in sperm. *Biol Reprod.* 1991a;44:375–381.

Suarez SS, Redfern K, Raynor PL, Martin F, Phillips DM. Attachment of boar sperm to mucosal explants of oviduct in vitro: possible role in formation of a sperm reservoir. *Biol Reprod.* 1991b;44:998–1004.

Yanagimachi R. Mechanisms of fertilization in mammals. In: Mastroianni L, Biggers JD, eds. *Fertilization and Embryo Development In Vitro.* New York: Plenum; 1981:81–182.

Yanagimachi R, Mahi CA. The sperm acrosome reaction and fertilization in the guinea pig: a study in vivo. *J Reprod Fertil.* 1976;46:49–54.