Sperm interaction with human oviductal cells in vitro

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In this article we describe the in-vitro interaction between human spermatozoa and oviductal epithelial cell monolayers. Freshly obtained spermatozoa were added to culture dishes containing human oviductal cells (co-culture), culture medium (control) or culture medium which had previously been used for culture of oviductal cells (conditioned medium). At 0, 5, 24, and 48 h of incubation the percentage of motile spermatozoa was determined and their motion characteristics analysed. Aliquots were taken to determine the percentage of acrosome-reacted spermatozoa. The spermatozoa were motile for a longer period in the presence of oviductal cells (54 ± 9% co-culture versus 18 ± 3% control, at 48 h) and the kinetics of the acrosome reaction exhibited a different pattern. In the control the percentage of reacted spermatozoa increased progressively throughout incubation. In co-culture, there was an increase only at 5 h; thereafter, the percentage of acrosome reactions did not change. Spermatozoa incubated in conditioned medium exhibited a behaviour halfway between the control and the co-culture. The pattern of sperm movement was not different in any of the experimental conditions. Although there was no binding between spermatozoa and oviductal epithelial cells, the frequency of the ciliary beat increased after spermatozoa were added to the oviductal cell monolayers. These results suggest that incubation with oviductal cells increases sperm survival, stabilizes the acrosome, and modifies the frequency of ciliary beat.

Key words: acrosome reaction/ciliary beat/ciliated cells/oviductal cell culture/sperm movement

Introduction

The oviduct is an organ that is essential to reproduction because it maintains the viability of the gametes, provides an adequate internal milieu for fertilization and early embryo development and delivers the blastocyst to the uterus at the right time for implantation (Hunter, 1988a). However, current knowledge about how the oviduct accomplishes these tasks is still limited.

During their passage though the oviduct, the spermatozoa probably do not swim freely in the oviductal lumen. In some domestic animals, including the rabbit, the pig and the cow, it has been reported that the spermatozoa bind to the oviductal ciliated cells (Motta and Van Blerkom, 1975; Fleckon and Hunter, 1981; Suarez, 1987; Suarez et al., 1991) and that this 'binding' may extend the fertilizing ability and the motility of the sperm cells (Nagai and Moor, 1990; Suarez et al., 1990; 1991; Pollard et al., 1991; Ellington et al., 1993; Gutierrez et al., 1993; Chian and Sirard, 1995).

Only a few studies, with inconsistent results, have been done regarding interaction between spermatozoa and oviductal epithelium in humans. Yeung et al. (1994) indicated that oviductal cells could maintain the motility of spermatozoa, and Kervancioglu et al. (1994) suggested that oviductal cells could induce the appearance of hyperactivated motility. Other studies, however, found no effect (Bastias et al., 1993) or only a marginal effect (Bongso et al., 1993) of the oviductal cells upon sperm movement characteristics. In a single report dealing with sperm capacitation during co-culture with oviductal cells (Bongso et al., 1993), it was found that the percentage of acrosome-reacted spermatozoa did not change during in-vitro culture. Recently, it was shown that the spermatozoa recovered from the Fallopian tube after in-vitro insemination were in close contact with the ciliated cells and the mucus-like secretions (Vigil et al., 1994). Pacey et al. (1995a) observed that human spermatozoa bound to the ciliated and non-ciliated epithelium of oviductal explants and that hyperactivated motility may assist spermatozoa in breaking this connection (Pacey et al., 1995b).

For the present study, we obtained human oviductal cells and cultured them in vitro. The resultant oviductal cell monolayers possessed functional ciliated and secretory cells. The effects of these cultures upon several characteristics of human sperm physiology are reported here.

Materials and methods

Chemicals

All reagents used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA), unless otherwise indicated.

Tissue acquisition

The procedures used to obtain the oviducts and transport them to the laboratory have been described previously (Vigil et al., 1994). Briefly, 13 Fallopian tubes were obtained from pre-menopausal patients with no clinical history of infection or neoplastic disease, who were scheduled for regular hysterectomies at the Hospital San José (Santiago, Chile). Ten of these patients (age range 38–48 years) were in the proliferative phase and three (age range 41–46 years) in the secretory phase of their menstrual cycle. No subject was recruited...
specifically for this study. Subjects signed a consent form allowing the use of their oviducts for research purposes. Once obtained, the oviducts were placed in a sterile receptacle on a filter paper wetted with phosphate-buffered saline at 37°C to avoid dehydration and damage to the organ. The oviducts were then cooled slowly during transport to the laboratory by placing the sterile receptacle on ice. The time between surgery and collection of oviductal cells was always less than 2 h.

Preparation of human oviductal ciliated cell monolayers

Once in the laboratory, the oviducts were placed on a dissection platform in a laminar flow sterile hood and freed of adherences, blood and cellular debris. The distal portion of the tube, the fimbria, was cut into pieces of 6-8 mm², rinsed in sterile Hank's solution and placed in minimum essential medium containing 5 mM EDTA for 40 min. Cells from the isthmus and ampullary regions of the oviduct were also obtained on one occasion. After this treatment, the ciliated epithelium was peeled off with forceps and placed in fresh Hank's solution. The sheets of epithelium were dispersed by passage through a 27-gauge needle several times. The dissociated epithelial cell suspensions were transferred to each well of a 4-well multidish (Nunc, Roskilde, Denmark) at a final concentration of 1-1.5 X 10⁶ cells in 1 ml of Waymouth medium supplemented with 20% heat-inactivated fetal bovine serum (FBS), plus 30 ml/l of a solution containing 7.5% sodium bicarbonate and 10 ml/l of penicillin-streptomycin solution. The 4-well multidishes had been previously coated with a 0.01% solution of collagen in acetic acid.

The oviductal epithelial cells were incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. After 5 days of culture, monolayers of epithelial cells had formed (65-75% confluence). The original medium was then replaced with 1 ml of fresh medium containing 5% FBS only. After 7 days in culture, oviductal epithelial cells had formed a confluent monolayer (100% confluence) with actively beating cilia.

Semen samples

Fresh semen samples were obtained from normozoospermic men according to the criteria laid down by the World Health Organization (1992). Spermatozoa were prepared on a discontinuous Percoll gradient consisting of 1 ml of 85% and 60% isotonic Percoll (Suarez et al., 1986). The gradients were centrifuged for 30 min at 300 g. The 85% bottom layer, containing selected motile spermatozoa, was recovered and washed for 20 min at 300 g with modified Tyrode’s medium supplemented with 2.6% bovine serum albumin. The sperm concentration was adjusted to 10 X 10⁶ cells/ml with Waymouth medium. The spermatozoa were added to the oviduct cells on day 7 of culture.

Experimental design

Before each experiment, the oviductal cell cultures were inspected, using phase-contrast optics and an inverted microscope (Nikon, Tokyo, Japan), to verify the active movement of the ciliated monolayer. The cultures were rinsed twice with 1 ml of Hanks’ balanced salt solution, pH 7.4, before adding 450 μl of Waymouth medium supplemented with 5% FBS. Control wells were those that contained culture medium alone, with no oviductal cells (control), or that contained spent culture medium that had previously been used to culture oviductal cells (conditioned medium). Conditioned medium was prepared by incubating 2 ml of culture medium (Waymouth medium + 5% FBS) with confluent cultures of oviductal cells. After 24 h at 37°C and 5% CO₂, the medium was removed, centrifuged for 5 min at 300 g, and used for the experiments. Then 50 μl of a sperm suspension was added to each culture well to achieve a final sperm concentration of 1 X 10⁶ cells/ml. Immediately after adding the spermatozoa (0 h), and after 5, 24 and 48 h of in-vitro incubation (at 37°C and 5% CO₂), the dishes were analysed using videomicrography for posterior sperm motion. The wells were placed on the heated stage of an inverted Nikon Diaphot microscope and then videotaped through a X10 phase-contrast objective with a working distance of 6 mm using a Sony Video Hi8 camera (Sony, Tokyo, Japan). The video image and a signal of the passage of time in 0.01 s intervals (For A, Los Angeles, CA, USA) were recorded by a Sony Video Hi8 cassette recorder. The percentage of motile spermatozoa and the quality of motility were determined at the time of recording by grading the forward progression according to WHO (1992) parameters. One hundred cells were counted in each experimental condition. At each sampling period, aliquots of spermatozoa from the co-culture and control groups were taken for assay of acrosomal status (see below).

Sperm motion analysis

Only continuous, intact areas of epithelium were analysed for motility of the sperm cells. The videotapes were analysed manually frame by frame as described previously (Katz et al., 1982, Morales et al., 1988). Twenty spermatozoa were analysed for each condition. Each spermatozoan was evaluated for straight-line velocity (VLS), linearity (LIN) of the trajectory, rolling frequency of the sperm head (RF), frequency (BF) and amplitude (AMP) of the flagellar beat, and flagellar curvature ratio (CR). A detailed description of these procedures has been published previously (Katz et al., 1982; Morales et al., 1988). To analyse the sperm interaction with the oviductal cells and/or secretion, five X40 fields were inspected for at least 1 min. Approximately 100 spermatozoa were observed in each field.

Detection of ciliary activity

The frequency of ciliary beat was monitored and recorded using microphotodensitometry, following the procedures developed by Villalón and Verdugo (1990). Sperm contribution to the signal would be minimal compared to 50-100 cilia per cell. The spectral structure of the light scattered fluctuations produced by the moving cilia was processed on line by a fast Fourier transform digital spectrometer. The averaged spectrum was recorded in an X-Y plotter.

Electron microscopy

Oviductal cell cultures, with or without spermatozoa, were fixed for scanning electron microscopy in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3). Samples were post-fixed in 1% osmium tetroxide in distilled water, dehydrated with a graded series of ethanol and then critical point-dried with CO₂ in a Bomar apparatus. The specimens were coated with gold and palladium before being observed with an ETEC autoscan scanning electron microscope.

Evaluation of sperm acrosome reaction

The procedure to evaluate the percentage of living acrosome-reacted cells has been published elsewhere (Cross et al., 1986; Morales et al., 1989). Briefly, spermatozoa were incubated with the supravital dye Hoechst 33258 and then fixed, permeabilized with ethanol and acrosome-labelled using fluorescein isothiocyanate–Pisum sativum agglutinin lectin (Vector Laboratories, Inc). The fluorescence of Hoechst 33258 and lectin was detected using an Olympus BH2 microscope. The percentage of live and reacted spermatozoa was determined counting a minimum of 200 cells.

Statistics

All percentages were subjected to arc-sine transformation before analysis. Bartlett’s test for homogeneity, followed by the F-test and then the paired t-test and/or Dunnett’s multiple comparison tests.
Figure 1. Scanning electron micrographs of monolayers of human oviductal cells taken 24 h after the addition of human spermatozoa. (a) A panoramic view of a monolayer of human oviductal cells after 8 days of culture (X700). (b) A higher magnification of the same culture: a ciliated cell, with a ciliary tuft and microvilli, is visible (X7000). (c) A spermatozoon can be seen in contact with an epithelial cell (X1300). (d) Another spermatozoon can be seen in contact with the epithelial cells (X3000). Note how the cilia surround the sperm tail. The arrows indicate the presence of mucus-like material.

were used to compare the number of motile and acrosome-reacted spermatozoa in the control and treated groups. Differences were considered significant at the 0.05 level of confidence. All results are given as mean ± SEM.

Results

Morphology of oviductal cells in vitro

After 1 day in culture, non-differentiated oviductal cells started to divide. After 3 days in culture these cells developed cilia. By day 7, the oviductal epithelial cells had formed a confluent monolayer. Two distinct cell types were observed in these cultures of oviductal epithelial lining: ciliated and secretory cells. Ciliated cells were generally polygonal in shape, flat and with well-defined contours. A cluster of 50–100 cilia per cell was usually found at the centre of the cell, with short microvilli covering the rest of the cell surface (Figure 1A and C). The presence of mucus-like material was also apparent in the cell cultures. Spontaneous ciliary activity measured in the cultured cells gave values of frequency of ciliary beat between 12 and 15 Hz. Similar values of ciliary activity have been reported in the human Fallopian tube in situ (Westrom et al., 1977).

After the cell cultures were inseminated, spermatozoa were rarely observed interacting with the oviductal cells or the mucus-like secretions (Figure 1A and B). Less than 5% of the total spermatozoa per high power field were seen in interaction with the epithelial cells at any given time, and this percentage did not change throughout the period of culture. This interaction consisted of a weak and transient (<10 s) contact or association between the spermatozoa and the epithelial cells (Figure 1C and D). These 'bound' spermatozoa were easily removed by agitation of the dish or gently pipetting of medium onto the cells. In addition, the contact was similar between spermatozoa and ciliated and secretory cells. Sperm behaviour in cultures derived from oviduct cells from the isthmic and ampullary region was not different from spermatozoa co-cultured with cells from the fimbria.

Ciliary beat frequency

The frequency of the ciliary beat of the human oviductal cells after 8 days of culture was 12.5 ± 1 Hz and increased to 15.5 ± 1 Hz 5 min after sperm addition (Figure 2). The ciliary beat frequency remained high for the next 50 min and then slowly decreased to stabilize at a value 8% higher than the basal frequency. In the absence of spermatozoa, the frequency of ciliary beating stayed constant for at least 2 h.

Sperm movement characteristics

Spermatozoa in co-culture with oviductal cells remained motile for up to 48 h (93 ± 4% at 24 h and 71 ± 3% at 48 h;
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Figure 2. Frequency of ciliary beat of human oviductal cells after 7 days of in-vitro culture. The frequency of ciliary beat was recorded in oviductal epithelial cells in culture before and after insemination with human spermatozoa (black circles). The arrow indicates when the spermatozoa were added. White circles depict the frequency of ciliary beat in cell cultures without spermatozoa. The data are presented as the percent increase over the basal value. A representative experiment is presented.

Figure 3. Percentage of motile spermatozoa at different times after in-vitro incubation with human oviductal cell monolayers. The white bars represent spermatozoa incubated with culture medium alone; the hatched bars represent spermatozoa incubated with human oviductal cells, the dotted bars represent spermatozoa incubated with conditioned culture medium. The results are the mean ± SEM of eight different experiments.

There were no indications of changes in sperm motility (hyperactivation) or attachment of the spermatozoa to the oviductal epithelium. There is abundant experimental evidence indicating that the oviduct provides the milieu in which gamete transport and survival, fertilization, and the first few days of embryo development occur (Hunter, 1988a). However, very little experimental work has been performed in humans, due to the logistic and ethical problems of an in-vivo study. Thus, an in-vitro approximation, as presented in this report, represents a logical and valid alternative. In the few reports which have concerned in-vitro culture of human oviductal tissue, however, the ciliary activity of the oviductal epithelia either was not assessed or was totally absent (Bastias et al., 1993; Bongso et al., 1993; Kervancioglu et al., 1994; Yeung et al., 1994). In the present study, we used a modified protocol to prepare oviductal epithelial cell monolayers. All the experiments reported here involved primary cultures of human oviductal cells in which cilia were actively beating. This may be an important point since it has been suggested that sperm attachment to oviductal epithelial cells plays an important role in maintaining sperm motility (boar: Nagai and Moor, 1990; Suarez et al., 1991; bovine: Pollard et al., 1991, Chian and Sirard, 1995; equine: Ellington et al., 1993; Thomas et al., 1994; ram: Gutierrez et al., 1993). Our data, however, showed that binding to oviductal ciliated or secretory cells may not be required to support sperm motility/viability. Human spermatozoa were rarely observed attached to ciliated or secretory cells, and when binding took place it was weak and transient. Similar observations were reported by Yeung et al. (1994). We also...
found that there were more motile spermatozoa in co-culture with human oviductal cells than in conditioned culture medium alone, and that spermatozoa incubated in control media alone exhibited the lowest percentage of motile cells. Together, these observations suggest that there are soluble as well as cellular components involved in maintaining sperm motility. The present results do not preclude, however, the possibility that, in vivo or under different in-vitro culture conditions, such as those reported by Pacey et al. (1995a), binding of spermatozoa to the oviductal cells may take place. In a previous study, where spermatozoa were recovered from the Fallopian tube a few hours after in-vitro insemination, binding between human spermatozoa and oviductal epithelial cells, the latter changed their pattern of ciliary beating upon contact with the spermatozoa. This is the first time that a spermatozoon-induced change in the pattern of ciliary beating has been reported. Mechanical receptors, located on the surface of the ciliated cells, may be responsible for the increase in ciliary beating (Villalón et al., 1989). Furthermore, in the respiratory tract, changes in ciliary activity after mechanical stimulus are propagated from one cell to neighbouring cells though gap junctions (Sanderson et al., 1988), allowing for dissemination of stimulatory signals through the epithelium. The physiological implications of this observation are unclear; however, several explanations are possible. The increase in ciliary beat frequency may be a mechanism that contributes to clear the oviduct of supernumerary, dead or immotile spermatozoa. In addition, it may represent a barrier against those spermatozoa incapable of vigorous swimming, preventing them from advancing toward the ampulla of the oviduct until they become capacitated and exhibit hyperactivated motility. Since the number of ciliated cells increases steadily from the uterine to the ovarian end of the oviduct, the magnitude of the barrier generated by the ciliary beat against further sperm progression increases along the tube.

In addition, it has been reported that contact between spermatozoa and oviductal monolayers may induce alterations in the sperm surface that may be involved in sperm capacitation and acrosome reaction (Hunter, 1988a,b; Suarez et al., 1990, 1991; Gutierrez et al., 1993). Ram spermatozoa co-cultured with sheep or hamster epithelial cell monolayers showed a significantly higher percentage of acrosome reactions than spermatozoa incubated with control media, conditioned media or IBRS-2 cells (Gutierrez et al., 1993). A study performed by Ellington et al. (1993) revealed that stallion spermatozoa co-cultured with oviductal epithelial cells underwent a higher percentage of acrosome reactions than spermatozoa in culture medium. In the present study, spermatozoa incubated with oviductal cells exhibited a sigmoidal pattern of spontaneous acrosome reactions, the maximum value being obtained at 5 h. In contrast, spermatozoa in control media showed a logarithmic pattern of acrosome reactions, and the maximum value was obtained at 48 h. Thus, our current data suggest that the
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