ANDROLOGY

Follicular Fluid Enhances Sperm Attraction and Its Motility in Human

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Purpose: Follicular fluid has a pivotal effect on motility and chemotaxis of spermatozoa for successful fertilization. The effect of human follicular fluid (hFF) and progesterone on attraction and motility of spermatozoa were investigated using simplified capillary assays.

Methods: Capillary tubes loaded with hFF, modified human tubal fluid (m-hTF), or m-hTF supplemented with progesterone, respectively, were used for assessments of attraction and motility of spermatozoa following culture at various time intervals.

Results: Number and motile ratio of spermatozoa in the tubes loaded with hFF were significantly ($P < .05$) higher than those with m-hTF. In the tubes loaded with m-hTF, m-hTF supplemented with progesterone, and hFF, the attracted number of spermatozoa were 34×10^5 , 131×10^5 , and 108×10^5 , and motile ratio of spermatozoa was 37, 48, and 82%, respectively.

Conclusions: We conclude that hFF clearly plays a crucial role in enhancing attraction and motility of spermatozoa, and progesterone has strong effect on attraction of spermatozoa.

KEY WORDS: attraction; follicular fluid; human; motility; spermatozoa.

INTRODUCTION

In order to achieve a consistent success in human in vitro fertilization (IVF) and artificial insemination (AI) programs, sperm motility and its concentration are essential (1,2). The ability of spermatozoa to undergo capacitation and acrosome reaction is also considered as one of the most important events to achieve successful fertilization (3).

Much progress has been made in understanding sperm chemoattractant and its components over the past few years. The chemoattractant includes peptides or protein with a molecular weight of 1–20 kDa, hormones such as progesterone, oxytocin, adrenalin, and receptors on the spermatozoa head, which are associated with the guanylyl cyclase enzyme and G proteins (4). Sperm transport in the oviduct is stimulated by the ovulatory process, in several species (hamster, 5; pig, 6; sheep, 7; cow, 6; human, 8). Large numbers of spermatozoa are detected in the oviduct ipsilateral to the ovulating ovary (sheep, 7; human, 9). Oocytes that are ovulated secrete chemotactic substances that might attract the sperm toward the oocytes (7). In vitro studies in human show that spermatozoal accumulation into follicular fluid is significantly higher than into simple medium and that chemoattractant effect of fluid from an individual follicle correlates with the fertilizability of the egg from the same follicle (8). In addition follicular fluid can alter the physiology and behavior of spermatozoa by increasing acrosome reaction and accelerating capacitation of the cell (10–15). This is due to many factors: physiological

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compounds, low molecular hydrophobic compounds (16), platelet activating factor (17,18), and progesterone (19,20). Chemical compounds in the follicular fluid are known to induce sperm motility and signaling interaction between sperm and oocyte during fertilization (8,21–23).

Progesterone causes sperm accumulation mainly by inducing hyperactivation-like motility and is known as not the major chemoattractant but a weak one (24). Out of several steroids found in human follicular fluid, only progesterone causes sperm chemotactic properties in dose-response curves (25,26) and increases the number of sperm cells to be chemoattracted (27).

The experiments described herein were undertaken to examine the effects of follicular fluid on the attraction of spermatozoa and its motility. The effect of progesterone on sperm attraction and its motility was examined by use of simply designed capillary tubes.

MATERIALS AND METHODS

Semen Preparation

The sperm concentration and motility of semen samples donated by five healthy volunteers were determined according to the provision of WHO (28). After liquefying at 37°C, the semen samples were washed in 10 ml of hepes-buffered human tubal fluid (H-hTF, 25) and centrifuged at $500 \times g$ for 10 min. The sperm pellet was resuspended in H-hTF and incubated in modified human tubal fluid (m-hTF) at 37°C in a humidified atmosphere of 5% CO₂ in air until used. Both H-hTF and m-hTF were purchased from Irvine Scientific (Santa Ana, CA).

Preparation of Human Follicular Fluid and Progesterone

Human follicular fluid (hFF) was collected ultrasonically by transvaginal-guided follicle aspiration from women participating in an IVF/ET program. In these women, multiple follicular development was induced by the pretreatment of a combination of FSH (Metrodin, Serono) and hCG (Profaci, Serono). The hFF was centrifuged at $2,000 \times g$ for 30 min. The supernatant was removed and divided into two groups: one half was inactivated at 56°C for 30 min, and the other not inactivated (control). Both fresh and inactivated hFF were filtered through with 0.22 μ m filter (Millipore, USA) and kept frozen at –20°C until used.

A stock solution of 2 mg/mL progesterone (Sigma, USA) in dimethylsulphoxide (DMSO, Sigma, USA)

was stored at –20°C in 0.25 mL aliquots. A working solution (200 μ g/mL) was prepared by diluting 1:10 in m-hTF.

Evaluation of Sperm Motility and Chemotaxis

Non-heparinized and autoclaved capillary tubes (i.d., 1.1 mm; o.d., 1.5 mm; length, 4.5 cm; Chase, USA) were sealed by heating at one end to prevent sperm from entering into the tubes. The tubes were loaded with hFF, m-hTF, and m-hTF supplemented with 200 μ g/mL progesterone, until 2 cm from the sealed end, respectively, and the remaining of 2.5 cm was loaded with m-hTF (Fig. 1). Loaded tubes were placed in 60-mm culture dish (Corning, USA) containing 10 mL of preincubated m-hTF, and 100 μ L of sperm suspension (final concentration of 100×10^6 cells/10 mL) was added into the dish near the sealed end of the tubes. The culture dish was then incubated at 37°C in a humidified atmosphere of 5% CO₂ in air.

The number of sperm attracted into the tube and its motility were assessed after 1, 2, and 4 h in culture. Briefly, after thoroughly mixing the suspension in the capillary tubes with a micropipetter, 10 μ L of sample was removed and placed on a Makler counting chamber (Sefi-Medical Instruments, Israel). The chamber was then placed on a stage of the inverted microscope (Nikon, Japan) warmed to 37°C. Ten fields were selected, and the number and motility of the sperm investigated. Only those spermatozoa moving in a straight and forward direction (rectilinear motility) were considered as a motile sperm. Five replicates were made from each donor.

Statistical Analysis

Differences among treatments were analyzed using one-way ANOVA after arc-sine transformation of the proportional data. Differences among treatments were evaluated by the chi-square test for the proportional data of motile spermatozoa, and by the student's *t* test for the number of attracted spermatozoa. Differences were considered significant when $P < .05$.

RESULTS

Effect of Human Follicular Fluid on the Attraction and Motility of Human Spermatozoa

Figure 2 compares the number of spermatozoa attracted into the tubes containing either m-hTF or hFF at different culture times of 1, 2, and 4 h, respectively.

Fig. 1. Schematic diagram showing tubes loaded with hFF, m-hTF alone, or m-hTF containing 200 $\mu\text{g/mL}$ progesterone were incubated in the 60-mm culture dish containing 10-mL m-hTF with spermatozoa pellet for 1, 2, and 4 h.

The number of spermatozoa attracted into the tube containing m-hTF after 1 h was considered as 100% for comparison with other treatments. The rate of spermatozoa attracted into the tube by increasing the culture time to 2 and 4 h tended to increase (132 and 121% vs. 100%, respectively). Furthermore, hFF attracted significantly higher ($P < .05$) number of spermatozoa than m-hTF. At 1, 2, and 4 h of culture, the numbers of spermatozoa attracted into

the tubes containing hFF were 300, 432, and 420%, respectively.

The ratio of motile spermatozoa out of the attracted proportion in the tubes at different culture times is presented in Fig. 3. In the tubes containing m-hTF, the ratio of motile spermatozoa at 1, 2, and 4 h of culture (36, 37, and 20%, respectively) were significantly lower ($P < .05$) than those of hFF (79, 84, and 78%, respectively).

Fig. 2. Relative ratios of total human spermatozoa attracted into tubes containing m-hTF or hFF after incubation for 1, 2, and 4 h. The percent total spermatozoa after 1 h incubation was considered as 100%. Alphabets a, b, and c represent percentages that are significantly different ($P < .05$).

Fig. 3. The percentage of motile spermatozoa attracted into tubes containing m-hTF or hFF for 1, 2, and 4 h. Percentages were calculated by a ratio of motile/total spermatozoa. Alphabets a, b, and c represent percentages that are significantly different ($P < .05$).

Effects of Treatment Methods of Human Follicular Fluid on the Attraction and Motility of Human Spermatozoa

To investigate the effect of treatment methods of hFF on the attraction and motility of spermatozoa, both fresh (control) and inactivated hFF were filtered through with 0.22- μ m syringe filter. The result was summarized in Table I. Although there was no significant differences ($P > .05$) between treatments, the control tended to increase the attraction of spermatozoa as compared with inactivated hFF.

Effect of Progesterone on the Attraction and Motility of Human Spermatozoa

Table II illustrates the number and the ratio of motile spermatozoa attracted into the tubes loaded with hFF alone, m-hTF containing progesterone, and m-hTF alone after incubation for 2 h. The number of spermatozoa attracted into the tubes was significantly different ($P < .05$) in the tubes loaded with m-hTF,

m-hTF containing 200 μ g/mL progesterone, and hFF (34×10^5 , 131×10^5 , and 108×10^5 cells, respectively). Out of the spermatozoa attracted, motile spermatozoa were significantly higher ($P < .05$) in tubes containing m-hTF, containing progesterone, and hFF than m-hTF alone ($63, 89$ vs. 12×10^5 cells). However, the percent of motile spermatozoa attracted did not increase by the addition of progesterone.

DISCUSSION

The results of this study demonstrated that human follicular fluid enhances attraction of human spermatozoa and its motility, and were similar to previous studies (8,26). Treating sperm with hFF before using them for IVF and AI clearly increased the rate of sperm motility. The addition of 20% of

Table I. Total and Motile Human Spermatozoa Attracted into Tubes Containing Fresh or Inactivated hFF After Incubation for 2 h

Treatment	No. of spermatozoa $\times 10^5$ (mean $\pm$ SEM)	
	Total	Motile (%)
Fresh hFF (control)	117.3 $\pm$ 8.3	101.5 $\pm$ 7.3 (86.5)
Inactivated hFF	112.2 $\pm$ 7.5	97.3 $\pm$ 6.5 (86.7)

Table II. Numbers of Total and Motile Human Spermatozoa Attracted into the Tubes Containing hFF, m-hTF Supplemented With Progesterone and m-hTF Alone After Incubation for 2 h

Treatment	Number of spermatozoa $\times 10^5$ (mean $\pm$ SEM)	
	Total	Motile (%)
m-hTF	33.7 $\pm$ 1.7 ^a	12.3 $\pm$ 2.05 ^a (36.5) ^a
m-hTF + progesterone	131.0 $\pm$ 6.9 ^b	62.9 $\pm$ 4.10 ^b (48.0) ^a
hFF	107.8 $\pm$ 6.9 ^c	88.5 $\pm$ 5.62 ^b (82.1) ^b

Note. Different superscripts in the same column indicate a significant difference ($P < 0.05$).

hFF into the medium resulted in an increase in the number of motile and hyperactivated spermatozoa (13,15,29). Although no direct comparison was made in the present study, Mendoza and Tesarik (15) reported that higher number of motile sperm was obtained in B2 medium supplemented with 20% hFF than B2 medium alone when sperm was cultured for 2, 6, and 24 h, respectively. Cohen-Dayag *et al.* (21) also suggested that spermatozoa needed chemotactic activity as a process of capacitation, and lose this responsiveness when the capacitated state was terminated. Furthermore, follicular fluid was reported to contain factors that stimulate motility and chemotaxis in spermatozoa.

Techniques in assessments of sperm chemotaxis and other process that cause sperm accumulation, such as trapping and chemokinesis, have been well reviewed by Eisenbach and Tur-Kaspa (30). To assess sperm chemotaxis and other process, several techniques have been introduced, such as ascending gradient, descending gradient, choice assays, and track analysis of swimming spermatozoa. In the present study, a modification of the capillary assay described by Ralt *et al.* (16) was used for sperm attraction. The capillary tubes loaded with an attractant, such as follicular fluid and progesterone, are immersed in a culture dish containing sperm suspension. They opined that this kind of assays cannot distinguish between chemotaxis and other processes that cause sperm accumulation.

Although the identity of the chemoattractants presented in human follicular fluid remains unknown, follicular fluid contains progesterone that could provide spermatozoa with increasing motility and attraction (16,26,29). In addition, decrease responsiveness to progesterone has been shown in the spermatozoa of oligozoospermic (11) and in infertile patients (29) as well, suggesting an alteration of putative sperm membrane progesterone receptor. Receptor of progesterone that is located on spermatozoa contains several domains, among which is one that has guanylate cyclase activity (4,31). Similarly the present study clearly shows that progesterone has a pivotal role on the attraction of spermatozoa. In contrast, it has been suggested that progesterone might be a weak chemoattractant to spermatozoa, but not a major chemoattractant in follicular fluid. Ralt *et al.* (16) reported that sperm attraction may be affected by several components rather than the concentration of progesterone in follicular fluid. They suggested that one of the components has a small (<10 kDa) nonhydrophobic molecule.

Although progesterone has a strong attraction to spermatozoa, the results of this study demonstrated that it did not affect their motility. This observation corroborates a previous report (32) that progesterone did not affect motility and acrosome reaction of spermatozoa. However, a possible candidate for the stimulation of mammalian sperm capacitation is the progesterone present in follicular fluid. This steroid initiates a rapid influx of extracellular calcium into the spermatozoa cytoplasm and induces acrosome reaction (19,20,33). Other factors such as glycoproteins and platelet-activating factor (PAF) have been reported to increase motility of spermatozoa (6,24,34).

Higher number of spermatozoa was attracted into the tubes containing fresh hFF than inactivated hFF but the difference was not significant. Chao *et al.* (2) reported similar results that hFF stimulates and prolongs the motility of human spermatozoa and suggested that it contains a heat-labile and nondialyzable active component(s). In a study of bovine spermatozoa, Ijaz *et al.* (35) also demonstrated that factors increasing sperm motility were present in oviductal epithelium cells, granulosa cells, and follicular fluid. These factors having ~8 kDa molecular weight were unstable to heat and could not transmit to protoplasm of the sperm. Moreover, it did degenerate under heat treatment of 100°C for 5 min but not at 55°C for 30 min.

The results in the present study suggest that hFF has beneficial effect on both attraction of human spermatozoa and its motility. In addition, a spermatozoa has strong attraction to progesterone, but did not increase its motility.

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