ANDROLOGY

Follicular Fluid Enhances Sperm Attraction and Its Motility in Human

BYEONG-GYUN JEON,1 JIN-SOO MOON,1 KWANG-CHEUL KIM,1 HYO-JONG LEE,2 SANG-YONG CHOE,2 and GYU-JIN RHO2,3

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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 $34 \times 10^5$, $131 \times 10^5$, and $108 \times 10^5$, 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...
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 × 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 × 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 μm 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 μg/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 μg/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 μL of sperm suspension (final concentration of 100 × 10⁶ 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 μL 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 arcsine 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.