Effect of human follicular fluid on sperm survival

A. Abu-Musa[1], A. Khalil[1], A. Hannoun[1], K. Sakhel[1], K. Takahashi[2]

[1]Department of Obstetrics and Gynecology, American University of Beirut, Beirut (Lebanon)
[2]Department of Obstetrics and Gynecology, Shimane Medical University, Izumo (Japan)

Summary

Objective: To study the effect of follicular fluid (FF) on sperm survival.
Method: Swim-up sperm suspensions obtained from 20 men with normal semen analysis were incubated with Ham's F-10 only and Ham's F-10 supplemented with 20% FF or 20% serum. Sperm motility was recorded every 12 hours for 72 hours.
Results: Sperm motility was maintained in all media for 48 hours. However, significantly more sperm samples remained motile at 72 hours in medium supplemented with FF and serum as compared to Ham's F-10 only.
Conclusion: FF has a positive effect on conserving sperm motility as a function of time.

Key words: Sperm survival test; Follicular fluid.

Introduction

Several tests have been developed to assess the fertilizing capacity of spermatozoa and to help predict the outcome of male infertility treatment in in vitro fertilization (IVF). Such tests include the sperm penetration assay, evaluation of the acrosome reaction, the hemizona assay and hypoosmotic swelling test [1, 2]. However, most of these tests are time consuming, require expensive laboratory equipment and highly trained laboratory personnel. The sperm survival test (SST), which was introduced by Fuse [3], correlates the length of sperm survival and fertilization rates. This test is simple and could be performed easily by any andrology personnel.

Follicular fluid (FF) is a dynamic medium rich in steroids, polypeptide hormones and growth factors. After ovulation the mixture of FF and peritoneal fluid accompanies the ovum and cumulus oophorus into the fallopian tube, thus constituting the final milieu for sperm/egg interaction and fertilization [4]. Although the incorporation of FF in assisted reproduction has been claimed to improve the clinical results [5, 6], the results of in vitro studies of the effect of FF on spermatozoa are not in agreement. Some authors observed inhibitory effects of FF on sperm motility [7, 8] whereas others reported stimulatory effects on both sperm motility and acrosomal reaction [9-11]. In this study, the effect of FF on SST was evaluated.

Materials and Methods

Sperm Collection and Processing

Semen samples were obtained from 25 men with proven fertility within two weeks of oocytes retrieval. Only sperm samples having the following characteristics were employed: >70 $\times$ 10^6 sperm/ml; >40% progressive motility at 30 minutes, and >30% normal forms [12]. The ejaculates, obtained after three to five days of abstinence, were allowed to liquefy for about 30 minutes at room temperature, then washed twice by centrifugation in Ham's F-10 medium (GIBCO, Grand Island, NY) at 300 $\times$ g. After the second wash, the supernatant was removed, and 0.3 to 0.5 ml of fresh medium was gently layered over the final pellet. At the end of one hour, a highly motile sperm fraction was obtained by collecting the supernatant. These "swim-up" specimens were analyzed for count and percent motility, and adjusted to 10 $\times$ 10^6 motile sperm/ml.

Follicular Fluid Collection and Processing

Follicular fluid specimens were obtained from patients undergoing oocyte aspiration for in vitro fertilization. The stimulation protocol in all patients included administration of 900 µ/day buserelin acetate nasal spray (Suprefact; Hoechst AG, Frankfurt, Germany) from day 21 of the previous cycle to the day of human chorionic gonadotropin (hCG) administration. Four ampoules of human menopausal gonadotropins (hMG; Humegon, Organon Ltd, Oss, The Netherlands) were administered daily as of the third day of the menstrual cycle. Patients were monitored by serum estradiol levels and transvaginal ultrasound scans. When two follicles reached a mean diameter of 18 mm, 10,000 IU hCG (Pregnyl; Organon Ltd) were given. Oocytes were scheduled 34-36 hours after hCG administration, using an ultrasound-guided transvaginal approach. Only fluid, without contamination of blood and obtained from follicles bearing mature oocytes that subsequently fertilized and cleaved, were used in this study. All samples were centrifuged at 1500 $\times$ g for 10 minutes immediately after oocyte recovery, heat inactivated at 56°C for 35 minutes, filter sterilized with a 0.2 µm filter, and stored at 4°C until used (maximum two weeks).

Blood samples were also obtained from the same patients on the day of hCG injection. After the blood was allowed to clot, it was centrifuged at 1500 $\times$ g for 10 minutes, heat inactivated, filtered and stored similar to FF.

Sperm Survival Test

The swim-up samples were divided into three aliquots: 1) FF was added to the sample at a dilution of 20%, 2) human serum was added to a dilution of 20% and 3) Ham's F-10 only (control). Spermatozoa were incubated for 72 hours and their motility was checked every 12 hours. The survival test was interpreted as positive if only motile sperm were present after a given incubation period and negative if only immotile sperm remained.

Statistical Analysis

The number of sperm samples that had positive motility at 24, 48 and 72 hours in the different culture media were compared using χ^2 analysis.

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Results

Table 1 shows the number of sperm samples with conserved motility as a function of time. Although the number of sperm samples incubated with FF tended to have more positive SST than other culture media, there was no significant difference between the different culture media. However, after 72 hours of incubation significantly more positive SST were noted in Ham's F-10 supplemented with 20% FF or serum as compared to Ham's F-10 only ($p < 0.05$).

Table 1. — *Number of swim-up samples with positive SST as a function of time.*

	+SST		
Medium	24h	48h	72h
Ham's F-10 + FF	24/25 (96)[a]	22/25 (88)	21/25[b] (84)
Ham's F-10 + Serum	23/25 (92)	21/25 (84)	20/25[b] (80)
Ham's F-10	22/25 (88)	20/25 (80)	12/25 (48)

[a]Values in parenthesis are percentages; [b]Significantly different than control, $p<0.05$.

Discussion

Along their way to the fertilization site, spermatozoa encounter various fluids secreted by the female genital tract including FF which accompanies the ovulated oocyte after follicular rupture into the fallopian tube. Follicular fluid is a dynamic medium rich in steroids, polypeptide hormones and growth factors. An increasing bulk of evidence indicates that FF and other fluids secerted by the female genital tract may influence spermatozoa function and subsequent interactions with oocytes. However, in vitro studies have produced heterogeneous and often conflicting results. Although some authors observed inhibitory effects of FF on sperm motility [7, 8], there is considerable evidence that human FF is a potent stimulator of human sperm capacitation and acrosome reaction [13, 14], sperm motility [9, 11] and hyperactivation [10, 15]. These partly contradictory findings can probably be explained by the biological variability of sperm and FF, and by the different experimental approaches employed to test the interactions between spermatozoa and these fluids. In contrast, the incorporation of FF in assisted reproduction protocols has been claimed to improve the clinical results [5, 6].

The results of this study show that the treatment of human sperm with human FF in vitro is able to conserve sperm motility as a function of time. It has been shown that the ability of spermatozoa to maintain their motility for an extended time is predictive of in vitro fertilization outcome [3, 16]. Although several tests are available to screen male infertility patients undergoing IVF, no single test or parameter has consistently proven to be the most useful. The advantage of SST is that it is easy to perform and more dynamic than other technically simple tests such as routine semen analyses and hypoosmolar swelling tests. It measures two important physiological events: the quality of sperm motion obtained after swim-up and sperm longevity [16]. Our data correlate with many other reports indicating that FF stimulates the acrosomal reaction [13, 14], sperm motility [9, 11] and hyperactivation [10, 15], improves the outcome of sperm penetration assay [17], and also enhances the pregnancy rates obtained in assisted reproductive technologies [5, 6]. All these effects are, in fact, either directly or indirectly coupled to sperm motility conservation or enhancement. This suggests that FF contains motility-conserving or-promoting factors able to rapidly interact with spermatozoa. Reports have pointed to the fact that FF, especially those obtained from stimulated cycles [18], contain high concentrations of agents (in particular steroids) able to promote sperm motility [10]. These agents may also be responsible for maintaining sperm viability.

Our study also demonstrated that the presence of serum in culture medium significantly prolonged the motility span of spermatozoa. Human serum has also been shown to stimulate hyperactivation of spermatozoa [13]. These findings might be expected since both FF and serum are steroid containing fluids, and, in addition, follicular fluid is similar to plasma in protein composition [19].

In conclusion, the addition of FF to culture medium was able to conserve sperm motility. This adds to the positive effects of FF seen in other sperm functions. It would be interesting to know the affect of FF and SST in patients with oligoasthenospermia.

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Address reprint requests to:
A. ABU-MUSA, M.D., Ph.D.
Department of Obstetrics and Gynecology
American University of Beirut
P.O. Box 113-6044/6A
Beirut (Lebanon)

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