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Sperm motility stimulation and preservation with various concentrations of follicular fluid

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Abstract
Purpose The purpose of this study is to evaluate the optimal concentrations of follicular fluid (FF) for sperm motility stimulation and preservation.
Methods Thirty normal semen samples and FF from women in an in vitro fertilization programme were used in an experiment. The semen was processed and incubated in Earle culture media with 20, 50, 80, and 100% FF (*v*/*v*) with controls. Sperm motility was evaluated and followed up for 48 h.
Results FF could stimulate progressive sperm motility at all concentrations and last for at least 12 h. However, at more than 50% *v*/*v* of FF, sperm demonstrated a rapid decline in progressive motility after 12 h of incubation compared to other concentrations and the control group.
Conclusion FF can stimulate the sperm motility properly at not more than 50% (*v*/*v*) concentration.

Keywords Follicular fluid · Human sperm · Motility

Introduction

Spermatozoa need to adapt themselves while in the female genital tract to be equipped for fertilization. The process of capacitation, hyperactivation, and acrosome reaction must be completed before the fertilization process. One essential factor in the adaptation process is the secretion and fluid in the female genital tract. The spermatozoa are induced to be adapted sequentially during the traveling from the vagina to the fallopian tube where the fertilization takes place [1]. The capacitation mainly occurs in the cervical canal and uterus with some role performed by the cervical and endometrial secretion. The hyperactivation and acrosome reaction mainly takes place in the fallopian tube with the assistance of the tubular fluid.

At ovulation, the oocyte–cumulus complex enters the fallopian tube along with the follicular fluid (FF). The follicular fluid appears at the time of fertilization indicating that there is some role of the fluid in the oocyte–sperm interaction. Previous studies have revealed the effects of the follicular fluid on the spermatozoa kinetics and properties [2, 3]. The hyperactivation and acrosome reaction process can be induced with various concentrations (20–100% *v*/*v*) of follicular fluid in vitro [4–9]. Therefore, follicular fluid stimulation of sperm motility might be applicable in either in vivo or in vitro use. This study aims to evaluate the effect on the sperm motility and longevity at different concentrations of follicular fluid.

Materials and methods

This study was taken at the Infertility Unit, Department of Obstetrics and Gynecology, Songklanagarind Hospital with the approval of the faculty's Ethical Committee from November, 2006 to January, 2007. Follicular fluid was voluntarily donated by women in an in vitro fertilization programme. The women were stimulated with the long protocol using buselerin acetate (Suprefact, Hoechst AG, Frankfurt, Germany) and follitropin alfa (Gonal-F, Serono, MA, USA) for superovulation. Only follicular fluid from

Follicular fluid can stimulate sperm motility optimally at not more than 50% *v*/*v* concentration.

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Table 1 Percentage of grade A plus B sperm motility at various concentrations of follicular fluid

	15 min	2 h	6 h	12 h	24 h	48 h
0% FF	65.53±16.6	62.43±16.2	58.21±17.2	55.33±19.9	49.37±19.3	30.03±18.6
20% FF	73.63±15.9*	73.57±16.1**	64.18±18.2**	53.40±21.7	51.33±19.9	24.90±18.9
50% FF	76.40±14.0**	77.47±13.7**	65.58±18.1**	53.30±21.1	44.53±20.7	18.17±17.1
80% FF	76.70±14.2**	75.11±12.0**	61.82±16.4	48.77±20.2	40.67±18.0	10.09±15.0
100% FF	76.83±14.8**	76.63±15.9**	62.41±17.2	38.43±19.9**	27.97±17.8**	5.97±8.1**

Values are means ± SEM (standard error of mean).
*$P<0.05$ as compared to the control in the same column.
**$P<0.001$ as compared to the control in the same column.

16 mm or bigger follicles with no blood contamination was taken for processing. The follicular fluid was then centrifuged at 500 g for 10 min and the supernatant was collected to be heated at 56°C for 30 min. The fluid was kept at −20° C for the experiments.

Thirty fresh normal semen samples were donated by 30 normal men via masturbation following the WHO criteria [10]. After examination, the semen samples were aliquoted equally into five parts. Each part was centrifuged at 500 g for 5 min and the supernatant was removed. Each pellet was filled with 1 ml of the following culture media; Earle culture medium (Biochrome KG, Berlin, Germany) supplemented with 10% human serum albumin (Sigma, St Louis, USA) as the control, the Earle medium with 20% FF, 50% FF, 80% FF, and 100% FF, respectively. All aliquots were incubated at 37°C under 5% CO_2 in air and were examined by two scientists for motility at 15 min, 2, 6, 12, 24, and 48 h sequentially. The semen analysis was performed manually with a Makler counting chamber (MidAtlantic Diagnostic Inc., NJ, USA). The examiners were blinded for the type of specimens. Motility grade A was defined as 'rapid forward progression' and motility grade B as 'slow forward progression'. Results were analysed with the SPSS V 12 program (Chicago, IL, USA).

Results

All 30 semen samples were processed and examined for the motility changes with the various concentrations of follicular fluid environment. Table 1 shows the motility grade A plus B of the spermatozoa at the different concentrations of follicular fluid with time change. All concentrations of FF can significantly stimulate motility of the spermatozoa when compared to the control. The stimulating effect can last for 6–12 h before a decrease in progressive motility. After 12 h, spermatozoa in 80–100% FF shows a sharp drop of progressive motility compared to the other groups.

Table 2 shows the grade A motility spermatozoa in FF and the control. Significantly, there are more grade A progressive spermatozoa in all concentrations of FF compared to the control. However, after 12 h, spermatozoa in 80–100% FF shows a marked decrease in motility similar to the results in Table 1.

Table 3 shows a decrease in percentage of the grade B motile spermatozoa in all FF concentrations compared to the control. The inter-observer correlation coefficient of the sperm motility examination is 0.89.

Discussion

The effects of follicular fluid on the spermatozoa have long been studied in both human and animal models. Follicular fluid has been shown to stimulate the progressive sperm motility and acrosome reaction in vitro. The findings indicate that follicular fluid plays a role in the adaptation process of the spermatozoa at the final stage before fertilization. The presence of follicular fluid along with the oocyte–cumulus complex at the ampulla where the

Table 2 Percentage of grade A sperm motility at various concentrations of follicular fluid

	15 min	2 h	6 h	12 h	24 h	48 h
0% FF	30.9±18.1	25.7±19.1	23.2±18.2	22.4±20.7	19.5±18.9	6.2±6.2
20% FF	47.3±26.2*	44.6±26.6*	36.1±20.2*	28.2±21.4*	21.9±18.9	6.6±6.9
50% FF	48.4±23.4*	44.4±26.9*	35.5±25.2*	28.1±21.1*	19.8±18.1	5.5±8.3
80% FF	48.2±20.1*	46.2±25.2*	35.4±20.2*	24.2±20.1	16.2±13.1	3.7±2.2*
100% FF	50.5±22.9*	51.5±26.4*	34.3±19.2*	19.9±18.9	12.1±14.3*	1.9±3.1*

Values are means ± SEM.
*$P<0.001$ as compared to the control in the same column.

Table 3 Percentage of grade B sperm motility at various concentrations of follicular fluid

	15 min	2 h	6 h	12 h	24 h	48 h
0% FF	34.6±12.1	36.7±14.8	35.5±16.0	32.9±18.3	29.9±16.1	23.9±15.9
20% FF	26.3±15.8*	28.7±15.2*	27.2±14.4*	26.2±13.8*	29.4±15.1	18.3±13.8*
50% FF	28.1±15.9*	33.1±19.9*	29.2±16.0*	25.2±12.5*	24.8±12.4	12.7±10.8*
80% FF	27.2±14.0*	29.1±18.0**	25.6±14.4**	21.8±11.8**	20.9±11.5	8.9±6.2**
100% FF	26.3±14.2*	25.1±15.4**	22.2±13.1**	18.5±11.1**	15.9±10.7**	4.1±5.6**

Values are means ± SEM.
*$P<0.05$ as compared to the control in the same column.
**$P<0.001$ as compared to the control in the same column.

fertilization takes place supports the concept. The spermatozoa must be hyperactivated and acrosome reacted in order to bind with the zona pellucida and fertilize the oocyte successfully.

Previous studies showed the stimulatory effect of the follicular fluid on the sperm motility. However, those studies used different concentrations of follicular fluid (10–100% *v*/*v*) to incubate the spermatozoa and with different times. Mendoza and Tesarik incubated spermatozoa in 20% follicular fluid and found a significant change in the progressive velocities of the motile spermatozoa after 6 h of exposure while that of the spermatozoa in FF had still preserved their motility after 24 h [4]. Kulin et al. [9] reported a significant change in motility only after a preincubation of spermatozoa in the capacitation medium for 3 h. Fabbri et al. [11] also reported a stimulatory effect when spermatozoa were incubated in 100% FF for 6 h. In contrast, some studies found no stimulatory effect but an inhibitory effect instead. Mortimer and Camenzind [7] found a progressive loss of motility of the spermatozoa after a 2-h exposure with a higher concentration of FF. Briton-Jones et al. [12] found a limited effect of FF on the poor quality cryopreserved human sperm. However, our study shows the stimulatory effect of follicular fluid at all concentration (20–100%) with no need for a preincubation period. The spermatozoa are induced to have more progressive, forward motility. The optimal concentrations for stimulation are 20–50% *v*/*v*. The stimulatory effect could last for al least 12 h before a rapid decline occurred.

Follicular fluid has been analysed to look for the active substances responsible for its effects on spermatozoa. Many forms of biological substances have been found in the fluid to affect the spermatozoa similarly, such as progesterone, peptides, antisperm antibodies or immunoglobulins, and hyaluronic acid [13–15]. The findings from those studies signify the complicated process with many substances from endosalpinx, oocyte–cumulus complex, granulosa cell, and serum working together to direct the fertilization. Although clinical application of the follicular fluid may be limited by its biological nature, the fluid may be applied in assisted reproduction technology to stimulate or select good spermatozoa for the procedures. Previous studies reported successful intrauterine insemination and in vitro fertilization programmes with follicular fluid use [16–18].

In summary, follicular fluid at 20–50% concentrations can stimulate active spermatozoa to become more rapidly progressive spermatozoa and maintain its effect for up to 12 h. However, further studies with the poor quality sperm and an analysis of the active substances in the follicular fluid are necessary to understand the role of the fluid and its applicability in clinical use.

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