USE OF HUMAN FOLLICULAR FLUID FOR IN VITRO SPERM ACTIVATION OF INFERTILE PATIENTS

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Abstract

Sperm activation in vitro was performed on 24 semen sample collected from 19 infertile patients . The study aimed to assure efficiency of human follicular fluid (hFF) in sperm activation by using centrifugation wash - out technique at 2000 rpm for 5 and 10 minutes.

The results showed significant decrease ($p < 0.001$) in recovery sperm concentration and significant improvement in sperm motility percentage ($p < 0.005$), ($p < 0.001$) in grade activity, leukocytes and phagocytes concentration and normal sperm morphology percentage after 5 min. of centrifugation period and activation. While the centrifugation period for 10 minutes caused insignificant increase in sperm motility percentage and significant improvement ($p<0.05$) in grade activity, ($p<0.001$) in leukocytes and phagocytes concentration and ($p < 0.005$) in normal sperm morphology.

The compared study between centrifugation period for 5 and 10 minutes showed insignificant differences of sperm parameters in two centrifugation period.

Introduction

It was known that the protein content as well as the presence of factors derived from biological fluid like plasma and human follicular fluid (hFF) influence spermatozoal characteristics mainly motility (Revelli, *et al.*, 1992) and acrosome reaction (Tesarik, *et al.*, 1993).

Some steroids in hFF may be present in concentration as 1000 folds greater than in plasma (Hartshorne, 1989). The principle FF steroids are oesteogen (E1), 17 - β estradiol (E2), 4-androstendion, testosterone and progesterone (Franchimont, *et al.*, 1989).

Hyperactivation of human spermatozoa performed to improved the sperm parameters , mainly motility. Active motility of the spermatozoa provides a vital tool may be correlated with capacitation, the acrosome reaction and fertility of human sperm (Maek, *et al.*, 1988).

In the present study , hFF was used in human sperm activation to evaluate the effect of steroid of hFF in activation and possibility for using this fluid instead of artificial media in sperm activation procedure.

Materials and Methods

Semen collection and processing :-

Twenty-four semen sample were obtained by masturbation from 19 patients after at least 2-4 days of sexual abstinence . Semen was collected into sterile wide-mouth plastic containers. The specimens were allowed to liquefy at 37°C for 20-30 minutes. The mean data of the semen analysis, including sperm concentration percent of motility, grade activity , leukocytes and phagocytes concentration and percent of

spermatozoa with normal morphology were recorded. The values these parameters considered as sperm parameters before activation.

Follicular fluid collection and Processing :-

Samples of FF were collected by vaginal laproscope under u/s scan from women undergoing treatment in the in vitro fertilization program in Saddam center for in vitro fertilization and infertility treatment . After examination of each aspirate for the presence of oocytes the FF was decanted into a round- bottom sterile culture tube and centrifuged at 3000 rpm for minutes . The supernatant was removed and transferred to clean culture tubes. All the FF samples were stored at- 20°C until use.

Sperm activation technique :-

One ml of liquefied semen sample was mixed with 2 ml of 5% dextrose supplemented with 20% inactive maternal serum. The mixture was centrifuged at 2000 rpm either 5 or 10 minutes at room temperature. After centrifugation, the supernatant was discarded and the final pellet was overlayered with one ml of hFF, kept in an incubator at 37°Cfor 30 minutes.

After incubation a drop from top part of hFF layer was aspirated by pipette and examined under 40X powerfield for record the sperm parameters after activation.

The results were analyzed by using t-test to indicate the level of significancy (Scheffer, 1980).

Results

The viability of sperm cells were assessed before and after activation by using centrifugation wash-out technique. Centrifugation at 2000 rpm for 5, 10 minutes and activation by using hFF caused significant improvement in sperm quality. The results for 5 minutes centrifugation are significantly decrease ($p<0.001$) in sperm concentration and significant improvement in sperm motility percent ($p < 0.005$), grade activity.

Table -1- Sperm Parameters before and after activation by using 5 minutes of centrifugation period

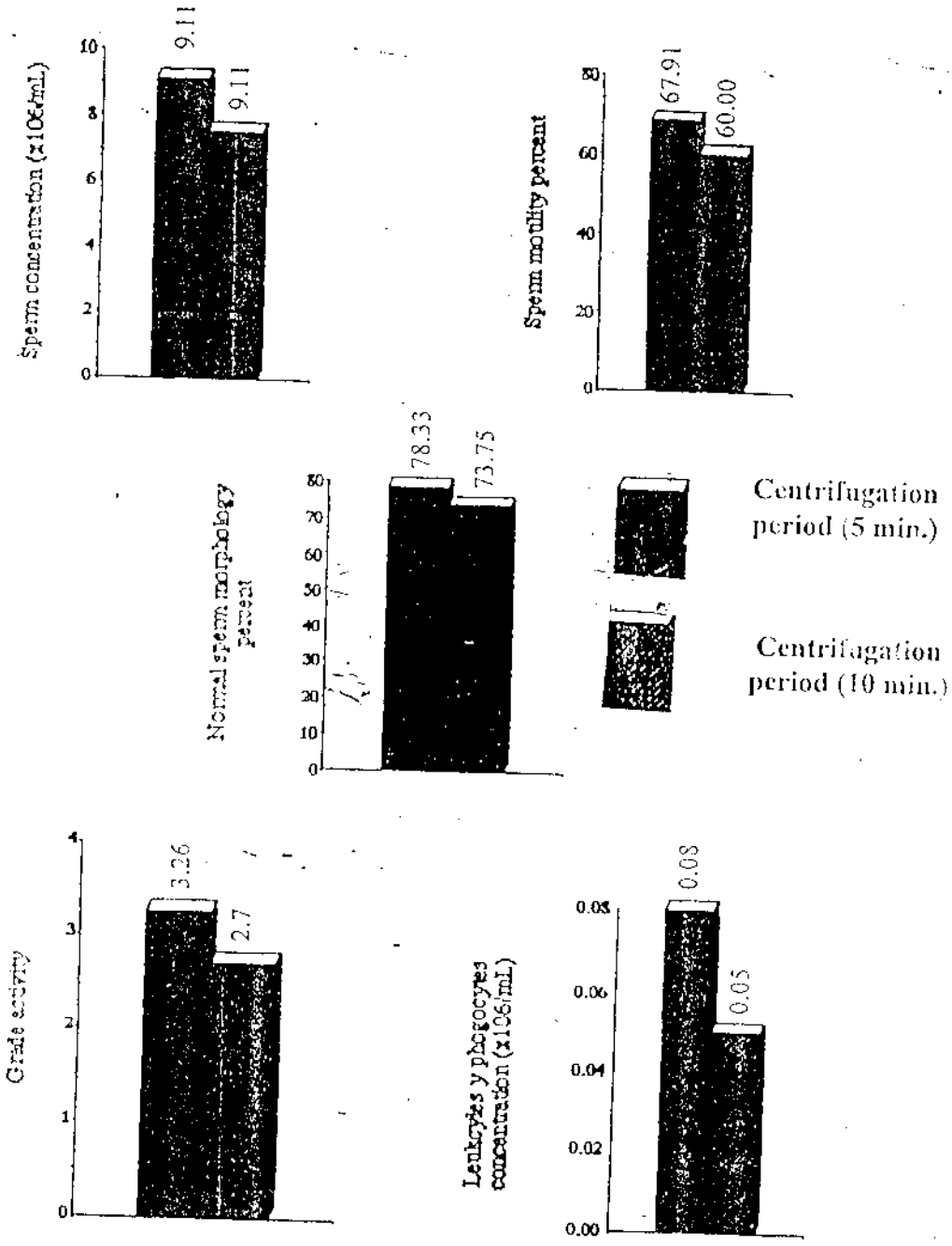
Mean of sperm parameter	Before Activation	After Activation
Concentration x 10^6 / ml	49.75 ± 21.86	9.11* ± 7.22
Sperm motility percent	39.83 ±18.76	67.91** ± 22.12
Grade activity	1.75 ± 0.61	3.26* ± 0.82
Leukocytes and phagocytic concentation x 10^6 / ml	3.83 ± 0.87	0.08* ± 0.01
Normal sperm morphology percent	52.08 ± 11.44	78.33* ±13.12

* $p < 0.001$ significant difference from before activation.
** $p < 0.005$ significant difference from before activation.

Table -2- Sperm Parameters before and after activation by using 10 minutes of centrifugation period

Mean of sperm parameters	Before Activation	After Activation
Concentration x 10^6 / ml	56.84 ± 26.60	7.50* ± 5.09
Sperm motility percent	44.41 ± 17.14	60.00 ± 21.11
Grade activity	1.88 ± 0.50	2.70* ± 0.67
Leukocytes and phagocytic concentration x 10^6 / ml	5.00 ± 1.02	0.05* ± 0.01
Normal sperm morphology percent	54.58 ±11.62	73.75 ≠ ± 15.52

* $p < 0.001$ significant difference from before activation.
** $p < 0.05$ significant difference from before activation.
≠ $p < 0.005$ significant difference from before activation.



≤0.05) in significant differences

Figure(1) Sperm parameters after activation by using 5 and 10 minutes of centrifugation period

<0.001), leukocytes and phagocytes concentration (p<0.001) and normal sperm rphology percent (p<0.001), (Table - 1 -).

Table 2 - revealed the results of sperm activation after 10 minutes of trifugation period. Also that is significant decrease (p<0.001) in sperm ncentration, and significant improvement in grade activity (p<0.05), normal sperm

morphology percent ($p<0.005$) leukocytes and phagocytes concentration ($p<0.001$), but insignificant increase ($p<0.05$) in sperm motility percent.

The compared study of the sperm parameters between 5 and 10 minutes of centrifugation period showed insignificant differences in all sperm parameters (Figure - 1).

Discussion

Follicular fluid surround the cumulus - Oocyte complex through all antral stages of follicular stages during follicular development. The steroid containing fluid s as serum or FF may stimulate hyperactivation of spermatozoa as compared with a control medium after the preincubation period (Kuhn, *et al.*, 1994).

Sperm behaviour incubated in FF was correlated with progesterone and 17-β estradiol in hFF (Fetterolf, *et al.*, 1991) . Progesterone induce acrosome reaction in human sperm by generating a rapid calcium influx into the cells . Another steroid in FF also may be help in hyperactivation of spermatozoa like estrogen and androgens . The concentration of testosterone in FF were significantly higher those undergoing gonadotrophin stimulation compared with spontaneous cycle (Chalib, 1995).

In this study, centrifugation wash-out technique demonstrated good efficiency of sperm activation. Centrifugation for 5 minutes and activation with hFF show a significant decrease in recovery sperm concentration and significant improvement in sperm motility percent, grade activity, leukocytes and phagocytes concentration and normal sperm morphology percent (Table -1-) . This results agreed with previous study of human sperm activation, by using centrifugation wash-out technique also at 400 x g and activated the sperm with Ham's F-10 media (Wong , *et al* ., 1986). The study revealed a significant decrease in sperm concentration and significant increase in percentage of motility, and sperm normality after washing. Also Kamad, *et al.*, (1993). documented that endothelin one of the important compound of FF which play an important role for significant increased of sperm velocity.

Treating sperm with hFF increase sperm penetration assay performance for some males, because hFF can induce acrosome reaction (Fukuda *et al.*, 1988) . Zinaman, *et al*, (1988), Suggested that using reproductive fluids like Peritoneal (PF) Uterine fluid (UF) or FF in sperm activation caused significant improvement in sperm motility compared to using of artificial media like Biggers , Whitten , and Whittingham (BWW)media.

The exposure of preincubated human sperm population to 50% FF only induces relatively small proportion of spermatozoa to undergo acrosome reaction in vitro (Mortimer& Camenzined, 1989).

Centrifugation period for 10 minutes and sperm activation, also caused significant differences in all sperm parameters except percent motility which showed insignificant increase (Table - 2-). This result agreement to the study of Ginsburg, et al. (1989), which revealed that centrifugation for 10 minutes at 3000 rpm and activation with Ham's F-10 media caused insignificant increase in sperm motility. The causes of this insignificant increase may be refer to the high pressure on the spermatozoa result from long period of centrifugation. Another study showed that FF has induced a significant increase in the percentage of eggs penetrated with sp activated by hFF (Blumenfeld & Nahhas, 1989).

It was concluded from present study that hFF has good efficiency in sperm activation specially in case of 5 minutes of centrifugation period.

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استخدام السائل الجريبي البشري في تنشيط نطف مرض مرضى العقم في الزجاج

الخلاصة

تم اجراء تنشيط النطف في الزجاج لـ ٢٤ عينة مني جمعت من ١٩ مريض يعاني من العقم. هدفت الدراسة الى معرفة كفاءة السائل الجريبي البشري في تنشيط النطف باستخدام تقنية الغسل والنبذ بقوة ٢٠٠٠ دورة/دقيقة ولفترة نبذ امدها ٥ و ١٠ دقائق.

بينت النتائج حصول انخفاض معنوي في تركيز النطف المسترجعة، وتحسناً معنوياً في كل من النسبة المئوية للنطف المتحركة ودرجة نشاط النطف وتركيز الخلايا القيحية والبلعمية والنسبة المئوية للنطف السوية بعد اجراء عملية النبذ لمدة ٥ دقائق. اما النبذ لمدة ١٠ دقائق فقد سبب زيادة معنوية في النسبة المئوية للنطف المتحركة ودرجة نشاط النطف والنسبة المئوية للنطف السوية.

لم تظهر الدراسة مقارنة بين فترتي النبذ ٥ دقائق و ١٠ دقائق أي فرق معنوي في قيم معالم النطف المدروسة.