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## Effects of Adding Human Follicular Fluid and Pentoxifylline on IUI Outcome of Asthenozoospermic Patients

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**Objective:** This study is a trial to improve the results of pregnancy rate following IUI by adding the follicular fluid (FF), and pentoxifylline (PX) to the culture medium used for preparation of asthenozoospermic semen samples.

**Subjects, Material and methods:** Ninety five infertile couples were involved in the current study (asthenozoospermic male partners with intact spouses) divided into three groups according to the sperm stimulants which were used for in vitro sperm activation followed by intrauterine insemination technique. Group one was FF-treated semen samples and consists of 40 couples, group two was FF+PX-treated semen samples and consists of 25 couples and lastly, group three was Ham's F-12 culture medium as a control group and consists of 30 couples. All the couples were followed up for determining outcome of pregnancy after IUI was done.

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# Effects of Adding Human Follicular Fluid and Pentoxifylline on IUI Outcome of Asthenozoospermic Patients

Saad S. Al-Dujaily<sup>α</sup>, Sabah M. Hussein<sup>σ</sup> & Sahar S. Raheem<sup>ρ</sup>

**Abstract- Background:** Although there are many motility stimulants have been found to play an important role in improving the active sperm motility and morphologically normal sperm (MNS) percentages when added *in vitro* with the culture medium for semen, the results of pregnancy rate following intra-uterine insemination (IUI) still not exceed 20%.

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**Results:** Out of the all 95 couples, 22 pregnancies (23.15%) were resulted following IUI. The best results were obtained by group one (FF treated group) in which out of 40 cases enrolled in this group, 12 pregnancies (30%) were resulted following IUI. Regarding group two (FF+PX treated group), out of 25 cases enrolled in this group, 5 women (20%) were pregnant following IUI. Regarding group three (Ham's F-12 treated group), out of 30 cases enrolled in this group, 5 women (16.67%) were pregnant following IUI.

**Conclusion:** Adding FF to culture media for *in vitro* sperm activation was associated with a higher pregnancy rate following IUI than other treated culture media.

## 1. INTRODUCTION

Infertility is the inability of a sexually active non-contraceptive couple to achieve pregnancy through one year<sup>(1)</sup>. It is a relatively common condition affecting approximately one in ten of the population. Infertility is either primary, when no pregnancy has ever occurred, or secondary, where there has been a pregnancy, regardless of the outcome. About 67 – 71%

and 29 – 33% of patients have primary and secondary infertility, respectively<sup>(2)</sup>. In half of these cases, a male factor is involved, making defective sperm function the largest single, defined cause of human infertility<sup>(3)</sup>. Asthenozoospermia is one of the major causes of infertility or reduced fertility in men<sup>(4)</sup>.

*In vitro* studies in human showed that spermatozoal accumulation into follicular fluid (FF) 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<sup>(5,6)</sup>.

Follicular fluid can also alter the physiology and behavior of spermatozoa by accelerating capacitation of the cell to induce sperm motility, increasing AR and signaling interaction between sperm and oocyte during fertilization. This is due to FF components which are a low molecular hydrophobic compounds, platelet activating factor and progesterone<sup>(7,8)</sup>.

On the other hand, certain metabolic stimulants induce sperm capacitation and AR via changes in the values of cyclic adenosine monophosphate (cAMP) and intracellular calcium ( $[Ca^{2+}]_i$ ). Examples of these stimulators are pentoxifylline (PX) and L-carnitine (LC)<sup>(9)</sup>.

Pentoxifylline is a methylxanthine derivative which inhibits phosphodiesterase, thereby elevating the concentrations of intracellular cAMP and/or cyclic guanosine monophosphate (cGMP). It is used in ART to enhance the function of inherently poor quality spermatozoa from oligoasthenozoospermic patients<sup>(10)</sup>. Other study does not suggest any increase in teratogenicity or evidence of congenital malformations in pregnancies following the using of PX in the media of IVF cycles<sup>(9)</sup>.

The Artificial Insemination (AI) is the first option treatment for infertile couples with cervical factor subfertility, mild to moderate male subfertility and unexplained infertility<sup>(11)</sup>. IUI, with or without controlled ovarian hyperstimulation, is one of the treatment modalities offered most often to subfertile couples because it is less stressful, invasive and expensive than other interventions such as *in vitro* fertilization and gamete intrafallopian transfer<sup>(12-13)</sup>. The goal of the present proposal is to examine the effects of adding human FF alone or in combination with PX on the

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medium prepared for *in vitro* sperm activation in intrauterine insemination (IUI) outcome.

## II. MATERIALS AND METHODS

This study was conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University through the period from September 2012 to June 2013.

Clinical examination was performed by a Consultant Urologist in charge of Male Infertility Unit in the Institute including presence or absence of varicocele, cryptorchidism, hydrocele, hernia and others. Alcohol consumption and smoking habits were also reported. Those patients were classified into three subgroups according to WHO criteria of seminal fluid analysis <sup>(1)</sup>. In this study ninety five infertile couples (asthenozoospermic male partners with intact spouses) divided into three groups according to the sperm stimulants which were used for *in vitro* sperm activation followed by intrauterine insemination techniques:

1. Group I: FF-treated semen samples. Include 40 couples.
2. Group II: FF and PX-treated semen samples. Include 25 couples.
3. Group III: Ham's F-12 culture medium as control group. Include 30 couples.

### a) Preparation of follicular fluid for *in vitro* sperm activation

Patients underwent oocyte retrieval 36 hr after hCG administration by transvaginal ultrasound guided follicular aspiration. The first aspirated follicle was usually the one with the largest diameter, and the first follicle allowed the collection of fluid without contamination of flushing medium. Specimens that were contaminated with blood were discarded. After removal of potential oocytes for treatment, the fluid was centrifuged ( $500 \times g$ ) to eliminate granulosa cells and to monitor the contamination of red blood cells and the clear supernatant was divided into aliquots and frozen at  $-20^{\circ}\text{C}$  <sup>(14)</sup>. The frozen samples were thawed and analyzed <sup>(15)</sup>. Solution of 20% hFF was prepared by the addition of 0.2 ml of hFF to 0.8 ml of Ham's F-12 medium.

### b) Investigations to assess the female reproductive status

Physical examination was done following the case history. The investigations were performed to assess the normal menstruation and ovulation. Hormonal assay was done to the female namely; early follicular estradiol ( $\text{E}_2$ ), FSH and LH level with serial vaginal ultra-sonographic examination for monitoring of follicular development and endometrial thickness. Their tubal patency were checked from the previous cycles by hysterosalpingography.

### c) Intra-Uterine Insemination Technique

IUI was performed when 0.5 – 0.75 ml of prepared semen was aspirated into 1 ml syringe attached to endo-cervical IUI catheter (Gynetics, Belgium). In IUI room, the spouse was prepared for insemination in lithotomic position. Non lubricated Cusco's speculum was placed in the vagina to visualize the uterine cervix <sup>(16,17)</sup>. The specimen was slowly ejected from the syringe with gentle grasping of the cervical lips to prevent escape of the solution out of the cervix then after few minutes the catheter was removed slowly. The patient remains on supine position for 30 minutes then she allowed leaving the theater. Following the day of insemination the patient instructed to take progesterone tablets (Duphaston® 10 mg/day) for two weeks as a luteal phase support. No use of antibiotic as it was detected that incidence of pelvic infection following IUI has been estimated to be less than 0.5% <sup>(17)</sup>. The diagnosis of pregnancy was done either by biochemical analysis (the detection of  $\beta\text{hCG}$  in blood after two weeks of insemination) or by sonographic examination to assess the pregnancy status

### d) Statistical analysis

The data of this study was analyzed by using SPSS (Statistical package for social sciences) versions 16 and Microsoft excel 2013. Numeric variables were expressed as mean  $\pm$  SEM whereas nominal variables were expressed as numbers and percentage. The analysis was done by using one way analysis of variance (ANOVA) to compare among different groups of *in vitro* sperm activation techniques. Also, descriptive analysis was done by using Chi-square depending on the nature of the data. Differences between values of means were considered statistically significant at ( $P < 0.05$ ) <sup>(18)</sup>.

## III. RESULTS

### a) Comparison of certain sperm function parameters after activation among IUI groups

In Table 1, there was no significant ( $P > 0.05$ ) difference in the mean of sperm concentration, active sperm motility (grade A, B and A+B) and the percentage of MNS between semen samples activated *in vitro* by Ham's F12 medium only (control) and other treated media.

**Table 1 :** Comparison of certain sperm function parameters among IUI groups following *in vitro* activation

Certain sperm function Parameters		Group1 IUI-FF-treated group (n=40)		Group 2 IUI-PX+FF-treated group (n=25)		Group 3 IUI-Hams.F-12 treated group (n=30)		P value
Sperm Concentration (X10 <sup>6</sup> /ml)		18.00	1.70	24.08	2.85	23.67	2.94	0.121
Active sperm motility (%)	Grade A	25.63	2.70	27.20	3.47	25.93	3.33	0.937
	Grade B	50.25	2.73	50.40	2.94	44.83	3.75	0.381
	Grade A+B	75.63	2.47	77.60	3.54	70.77	4.56	0.399
Morphologically normal sperm (%)		60.38	2.14	57.20	2.62	60.20	3.96	0.721

Values are expressed as mean  $\pm$  SE. ANOVA Test

PX: pentoxifylline

FF: follicular fluid

b) The results of pregnancy rate after *in vitro* sperm activation

Tables 2 and 3 reveal the effect of three different sperms stimulants (FF, FF+PX and Ham's F-12) on pregnancy rate and percentage (%) following IUI. Out of the all 95 couples, 22 pregnancies (23.15%) were resulted following IUI. The best results were obtained by group 1(FF treated group) in which out of 40 cases enrolled in this group, 12 pregnancies (30%) were resulted following IUI. Regarding group 2 (FF+PX

treated group),out of 25 cases enrolled in this group, 5 women (20%) were conceived following IUI. Regarding group 3 (Ham's F-12 treated group), out of 30 cases enrolled in this group, 5 women (16.67%) were conceived following IUI.

There was a significant ( $P < 0.05$ ) difference in the rate of pregnancy between group 1 and group 2 (table 4-21), while there was no statistically significant ( $P > 0.05$ ) difference in the rate of pregnancy between group 1 and 3.

**Table 2 :** Comparison of pregnancy rate following IUI after *in vitro* sperm activation between group 1 and group 2

Status of pregnancy		Pregnancy Rate			
		Group 1 (FF treated)		Group 2 (FF+PX treated)	
		No.	%	No.	%
Pregnant	Yes	12	30	5	20
	No	28	70	20	80
	Total	40	100	25	100
$\chi^2 = 9.474$					
DF = 1      P < 0.05					

**Table 3 :** Comparison of pregnancy rate following IUI after *in vitro* sperm activation between group 1 and group 3

Status of Pregnancy		Pregnancy rate			
		Group 1 (FF treated)		Group 3(Ham's F-12 treated)	
		No.	%	No.	%
Pregnancy	Yes	12	30	5	16.67
	No	28	70	25	83.33
	Total	40	100	30	100
$\chi^2 = 1.656$					
DF = 1      P > 0.05					

## IV. DISCUSSION

The current study demonstrates that IUI by a sperm washed *in vitro* using a medium supplemented with FF results in a higher pregnancy rates per cycle (30%) in asthenozoospermic patients than by using Ham's F-12 medium alone (16.6%) even the results of certain sperm function parameters after *in vitro* preparation did not reveal any significant between them. This finding can be explained by the fact that the extent of hyperactivated motility produced by FF is positively correlated with the extent of zona binding, the acrosome reaction, zona-free oocyte penetration and fertilizing capacity *in vitro*<sup>(19)</sup>. Additionally, human FF is an efficient capacitating agent and only the capacitated sperm are able to migrate towards the ovum site, under thermotaxis and chemotaxis stimuli<sup>(20,21)</sup>.

It has been reported that FF may modulate spermatozoa function to be indicative of pregnancy outcome<sup>(22)</sup>, this fact can verify and confirms the original results.

The contents of FF also play a crucial role in the pregnancy outcome. A lower activity of the conversion enzymes, ovarian 11 beta hydroxy steroid dehydrogenase (HSD11 $\beta$ ) which is found in FF interconverts 'active' cortisol to 'inert' cortisone, have been associated with positive pregnancy outcomes for patients undergoing ART<sup>(23)</sup>.

In this study, there was a significant ( $P < 0.05$ ) difference in the rate of pregnancy between IUI- FF treated group (pregnancy rate was 30%) and IUI-FF+PX treated group (pregnancy rate was 20%). This finding suggests that IUI following *in vitro* sperm activation by FF containing culture media is associated with a higher pregnancy outcome than FF+PX containing media. Moreover, the proper time of IUI that performed enclosed with ovulation time can lead to increase in pregnancy rates, and is best seen in patients with pure andrological indication. At the same time, careful selection of patients might play part in achieving success with IUI. Furthermore, the use of fertility drugs like gonadotropins in the controlled ovarian stimulation followed by IUI provide better pregnancy rate<sup>(24)</sup> than the using of other ovarian stimulants.

The current study found that pregnancy rate of IUI following FF+PX (20%) was approximately similar to the results of Al-Dujaily *et al* (20.8%) using PX alone<sup>(25)</sup>. Both results had no significant different in pregnancy outcome than the pregnancy outcome following *in vitro* sperm activation with FF alone. This suggest that there is no further advantage to add PX to FF-containing culture media for the enhancement of active sperm motility, morphologically normal sperm percentages or pregnancy rate following IUI.

Thus the present study concluded that adding the FF to medium used for IUI can enhance the results of pregnancy rate of infertile couples.

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