Effect of Pentoxifylline on the Outcome of Artificial Inseminations

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ABSTRACT:

OBJECTIVE:

To highlight the effect of Motility Stimulant ***Pentoxifylline*** (PF) used to activate semen samples when managing couples suffering from primary Infertility due to either unexplained reasons or mild male factors by Artificial Insemination.

DESIGN: Prospective study.

SETTING: Institution of Infertility Treatment and Embryo Research, Al-Nahrain University, Baghdad -Iraq.

METHODS:

Ninety four couples suffering from mild male or unexplained primary infertility were involved in this study. According to results of Postcoital test (PCT) performed to the participants prior to Insemination, they were divided into two groups. Group 1 (67 couples) women with positive (+ve) PCT (>5 motile sperm /HPF), they underwent alternative cycles of Intra- uterine and Intra-cervical Inseminations (IUI) (ICI). Group 2 (27 couples) those with negative (-ve) PCT (< 5 motile sperm/HPF), they were inseminated by intra-uterine method only. The couples underwent 120 insemination cycles. PF was added following sperm washing in 81 inseminations. Where as 39 inseminations were performed after sperm washing technique only.

RESULTS:

The study recorded 18% pregnancy rate (17 out of 94 women). IUI was done in 72 cycles in which PF was added following sperm washing in 48 cycles resulting in 10 pregnancies (20.8%). While only two women conceived form 24 IUI cycles without using PF (8.3%). This difference is statistically significant ($P<0.05$). On the other hand, ICI was done in 48 cycles; PF was used in 33 cycles successfully resulting in 5 pregnancies (15.15%). Whereas , no pregnancy recorded when PF not used in the remaining 15 ICI. PF was used in 8I insemination cycles treated by IUI or ICI had resulted in 15 pregnancies (18.5%). This result is significantly higher ($P<0.05$) than the recorded pregnancy rate of the control group in whom 39 inseminations with IUI and ICI done without using PF (2/39, 5.1%).

CONCLUSION:

Pentoxifylline improves pregnancy rate when used to activate sperm function in both Intrauterine and Intracervical Inseminations.

KEY WORDS: Artificial Insemination, Pentoxifylline.

INTRODUCTION:

Sperm motility is one of the most important prerequisites to achieving fertilization and pregnancy. The sperm must cross a great distance *in vivo* through the barriers of the reproductive tract to the site of the ovulated oocyte. The development of assisted reproductive techniques (ART) has allowed many infertile couples to conceive. These include Artificial Insemination (AI), *In vitro* fertilization (IVF) and intra cytoplasmic sperm injection (ICSI) [(1)].

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Artificial Insemination involves the placement of sperms within the female genital tract, either intra-uterine (IUI), intra-cervical (ICI), intra-vaginal, intra-peritoneal or intra-tubal [(2)]. In IUI, the cervical canal is passed and the washed spermatozoa are directly deposited into the uterine cavity. Previously, IUI was done by transferring an aliquot of the ejaculated semen or the first portion of a split ejaculate. This technique was found to be hazardous because the prostaglandin contained in the semen can cause uterine cramping and moreover bacterial contamination could be resulted in some cases [(3)]. The refining of IVF techniques has demonstrated the important role that the culture medium plays in maintaining ova and sperm viability and facilitating fertilization. So many centers use IVF culture medium to prepare motile sperms for IUI [(4).]

In addition many substances have been proposed for the stimulation of human sperm motility such as methylxanthine(e.g. caffeine and pentoxifylline), gonadotropin hormones FSH, LH, and E_2 [5, 6,7]. Pentoxifylline is phosphodiesterase inhibitor; inhibit the break down of cyclic adenosine monophosphate (cAMP). Intracellular cAMP levels are known to play a central role in sperm motility by activating cAMP-dependent protein Kinase (PKA), an important modulator of sperm capacitation [8]. The goal of this study is to compare the two techniques of Artificial Insemination using washed husband semen with the motility stimulant substance (pentoxifylline) as a modified method for the treatment of infertility.

MATERIALS AND METHODS:

This study was conducted at the Institution of Embryo Research and Infertility Treatment, University of Al-Nahran, during the period from the 1st October 2004 till the end of August 2005. Ninety four couples with primary infertility due to unexplained reasons or relatively mild male factors (sperm conc. >or = 15 million, sperm motility > or = 35% grad a+b, morphologically normal sperm > or = 30%) were selected to participate in this study. Their duration of infertility ranged between 3-11 years. **1. Female investigations:** The average age of those women ranged between 22 and 40 years. All of them were apparently normal and were further assessed and investigated by a gynecologist. They have a normal menstrual cycle and ovulation which were confirmed by the serial vaginal U/S and mid luteal phase progesterone level. Their tubal patency is checked by HSG and /or laparoscopy. Early follicular phase FSH, LH, E_2 level and mid cycle E_2 was also done as part of the workup. **2. Males investigation:** All the husbands included were examined by a consultant urologist in the Institute. They were apparently normal and their **Seminal Fluid** analyzed by a macroscopic and microscopic examination using the standardization of WHO [9].

3. Post coital test and cervical mucus score: Post coital test (PCT) was performed to the wives before the insemination cycle and prior to ovulation as determined by the vaginal ultrasound and serum estradiol (E_2) level. A sample of mucus was aspirated from the endocervical canal using a modified catheter attached to 1ml insuline disposable syringe and examined directly for the cervical score and the number of progressive motile sperm (Ga+b), 5 or more progressive motile sperms are regarded as good or +ve PCT, less than 5 motile sperms are regarded as poor or –ve PCT. According to the result of PCT, the patients were divided into 2 groups Figure 3-2:

1. Group 1, with good PCT (n=67), they underwent alternative cycles of intrauterine (IUI) and intra-cervical inseminations (ICI).

2. Group 2, with poor PCT (n=27), managed by IUI only.

4. Preparation of Pentoxifylline:

A pentoxifylline powder (Sigma chemicals, Germany) was added to a universal IVF medium (MediCult, Denmark) to prepare a solution every week in a concentration of 1 mg / ml (3.6 mMol/L) and kept in the refrigerator (1-6c°) until use [10, 11].

5-*In vitro* sperm activation technique:

The semen sample was collected in a special room in the theater by masturbation in a clean, dry and sterile disposable Petri-dish after 3-7 days of abstinence, and then transported to the laboratory immediately and placed in the incubator at 37 c° till complete liquefaction. The volume is measured by a graduated centrifuge tube, a drop of semen sample was put on slide and covered by a cover slip and examined under a light microscope at the magnification of 40 X power for the following parameters: **1.** Sperm concentration. **2.** Sperm motility percentage. **3.** Grade activity of progressive forward movement (a+b). **4.** Morphologically normal sperm. **5.** Round cells count. **6.** Agglutination. **7.** Others. Then each specimen was washed with a suitable volume of IVF media and centrifuged for 10 minutes at 1600-2000 run per minute (rpm). The supernatant was discarded and the pellet was re-suspending with media containing pentoxifylline at a concentration of 1mg/ml and incubated at 37c° for 30 minutes. After this medium was added to take the volume up to a total of 5ml, the sample was then centrifuged. The sperm pellet was re-suspended in the medium and kept in the incubator till use. The suspension used for insemination [11]. A drop from the supernatant was examined under the microscope and the result was recorded.

6- Program of insemination: Gonadotrophins (pergonal®75 IU) were used for ovarian stimulation in a dose of 75-150 IU /day starting from cycle day 2. The follow up was done by Vaginal ultra-sound to monitor number and size of the ovarian follicles and the endometrial thickness. When size of the follicles reaches 17-18 mm, the ovulating dose of HCG (Pregnyl®) (5000 IU-10000 IU) was injected. Insemination usually done 24-36 hours after the HCG injection.

7-Insemination technique:

In a lithotomy position, a non lubricated Cusco's speculum was put in the vagina to visualize the uterine cervix. A special intra-uterine catheter connected to a syringe containing 0.3-0.5 ml of a prepared activated sperm sample introduced inside the uterus.

For ICI, the catheter was only introduced inside the cervix. The washed semen sample then pushed slowly and steadily. After 5 minutes wait, the catheter was released gradually. For IUI, the catheter was introduced till it reaches the uterine fundus then the sample pushed slowly and steadily. After the insemination, the patient often stays in the insemination room for at least 30 minutes without mobilization. **8. Luteal phase support:** Start from the next day after insemination by using progesteron tablet (Duffaston® 10 mg /day) for 2 weeks then a blood sample was obtained from the patient to test β-HCG level. **9. Statistical Analysis:** The data was shown as mean ± SEM. The results were statistically analyzed using the Student's *t*-test and Chi-square depending on the nature of the data. When P value reaches the 0.05, the result was considered significant [12].

RESULTS:

1. Effect of Pentoxifylline (1mg /ml) on sperm function parameters following i*n vitro* activation technique: The couples underwent 120 inseminations Cycles , in 81 cycles pentoxifylline was used in the sperm washing procedure and the remaining 39 cycles sperm washing was done without it.

The effect of pentoxifylline (1mg/ml) on the sperm function characters following *in vitro* activation technique was shown in table (1). There was a significant (P<0.001) decrease in sperm concentration (million/ml) following *in vitro* activation with PF compared to that before activation. A significant (P<0.001) increase in the percentage of motile sperm was observed following the activation with PF compared to that before activation (88.11 ± 0.7 versus 44.5 ± 1.3). The grade activity of forward progressive movement (a+b) following *in vitro* sperm activation with pentoxifylline was significantly (P<0.001) higher than that before activation (74.34 ± 0.75 versus 24.3 ± 1.09). The mean of morphologically normal sperm after washing with PF was significantly (P<0.001) improved compared to that before activation (77.5 ± 0.99 versus 42.68 ± 1.06).

Table 1: Effect of adding pentoxifylline (1mg/ml) on sperm function parameters Following i*n vitro* activation technique.

Sperm function parameters	In Vitro Activation	
	Before activation	After activation*
Sperm concentration (million /ml)	32.43±1.4	15.2±0.53
Sperm motility (%)	44.5±1.3	88.11±0.7
Grade Sperm motility a+b (%)	24.3±1.09	74.34±0.75
Morphologically normal sperm (%)	42.68±1.06	77.5±0.99

Values are mean ± SEM
Total No. samples = 81
* P< 0.001: significant difference from before activation.

2. Effects of Pentoxifylline on sperm function parameters of asthenospermic patient:

A comparison of different sperm function parameters between the group which used PF in sperm activation for insemination (n=81) and the control group in which sperm activation was done without adding PF (n=39) is shown in a histogram 1. The sperm concentration (million/ml) is significantly more (P<0.025) in the PF treated group (15.2 ± 0.53 versus 13.12 ± 0.66). Also for sperm motility percentage, it was significantly (P<0.025) higher in the PF treated group than in the control group (88.11% ± 0.74 versus 84.79% ±1.52). A significant difference (P<0.025) in the grade a+b motility percentage was also observed (74.34% ± 0.74) in the PF treated group compared to (69.97% ± 1.8) in the control group. The morphologically normal sperm (%) was significantly increased (P<0.025) in the PF treated group (77.5 ± 0.99) compared to the semen samples without PF (66.15 ±1.7).

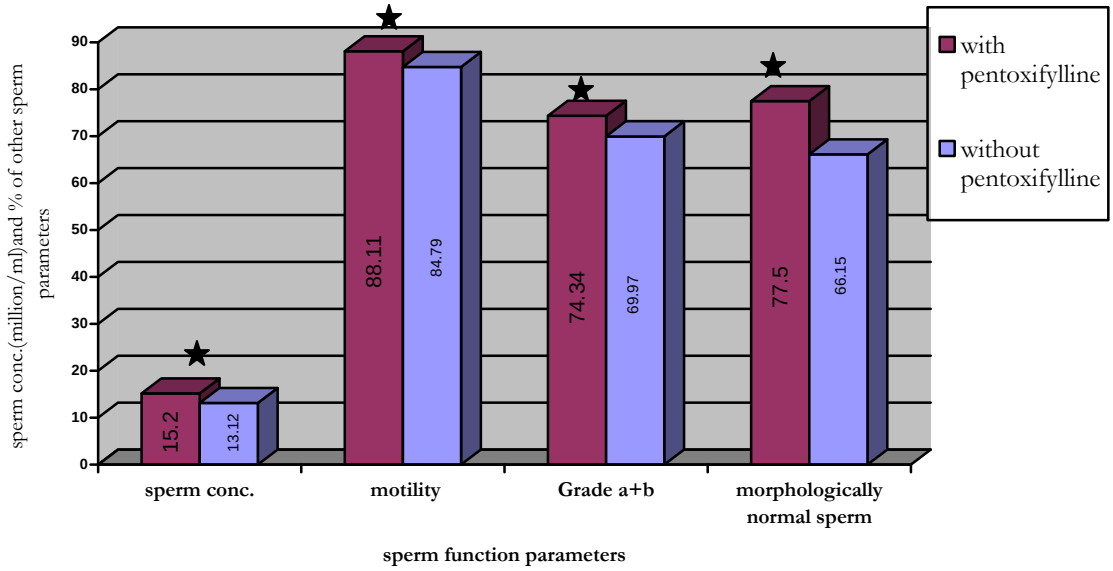

Figure (1): The effect of using pentoxifylline in sperm preparation samples on different sperm function parameters of asthenospermic patient.

Values are the mean± SEM
No. Semen samples with pentoxifylline=81 No. Semen samples without pentoxifylline =39
★ P < 0.025: significant difference from control group (without pentoxifylline).

3. Pregnancy rate following IUI and ICI with and without adding PF:

During this study, 17 out of 94 couples conceived (18%). The pregnancy rate per cycle was shown in table (3). Pentoxifylline was used in the sperm washing of 48 cycles out of 72 cycles of IUI.

There were 10 cases of pregnancy (20.8%) in this group and only 2 pregnancies out of 24 in the control group (8.3%), this difference was statistically not significant (P>0.05). For ICI, 33 cycles that used PF in sperm preparation for insemination resulted in 5 pregnancies (15.15%) and 15 cycles as a control group (sperm preparation without PF is used for insemination), no pregnancy resulted, but also this difference is statistically not significant (P>0.05). The pregnancy rate per IUI cycle as a whole (12/72) 16.7% is greater than the pregnancy rate per ICI cycle (5/48) 10.4%, but this difference is statistically not significant (P>0.05). Using pentoxifylline in the sperm preparation for insemination in a total of 81 cycles resulted in 15 pregnancies. The pregnancy rate was 18.5% is significantly (P<0.05) higher than the control group (39cycles) in which sperm activation without pentoxifylline is used (2/39, 5.1%). The diagnosis of pregnancy was done either by a biochemical or by ultrasound. Two of the pregnancy case ended by a delivery of a full term healthy infant and three of them are still pregnant, two with single fetus and one with twin. Regarding the other pregnant patients, we do not know their fate due to the bad communication with the patients and lack of information.

Table 3 : Pregnancy rate following IUI and ICI with and without Adding PF.

Method of insemination	Pregnancy rate per cycle (adding PF)	Percentage (%)	Pregnancy rate per cycle(without PF)	Percentage (%)	Total no.	Percentage (%)
Intrauterine insemination IUI	10/48	20.8	2 /24	8.3	12/72	16.7
Intracervical insemination ICI	5/33	15.15	0/15	0	5/48	10.4
Total	15/81	• 18.5	2/39	• 5.1	17/120	14.2

Total No. cycles of IUI without PF = 24 Total No. cycles of ICI without PF =15
Total No. cycles of ICI with PF =33 . P< 0.05: significant difference between the two groups.

DISCUSSION:

Using PF to mild Asthenospermic semen had improved sperm motility markedly and probably via the role in protecting the sperm plasma membrane, such achievement is critically useful in the process of semen preparation in Assisted Reproduction Techniques. Because sperm motility "as well known" plays an extremely important role in positive results of the techniques. Yovich *et al.* [11] and Tarlaziz *et al.* [13] recognized an improvement in the IVF rate and showed a promising development in their studies when PF added to the media. Moreover, Yovich *et al.* [14] found an increase in pregnancy rate following PROST program (pronuclear stage tubal transfer) for severe male factor infertility when PF is used. Furthermore pentoxifylline has been successfully used as a pretreatment to stimulate epididymal and testicular sperm motility for ICSI [15]. The ladies included in this study were inseminated either intrauterinally or intracervically. For those with IUI, the deposition of a high concentration of motile sperm directly in the endometrial cavity would have a rational basis in case of male factor infertility, because of Oligospermia or Asthenospermic.. It would also seem logical in case of cervical factor infertility, in which the cervical mucus may act as an abnormal barrier to sperm penetration and migration for a variety of reasons [16]. Because the fertilizing life span of ovum is estimated to be 12 hours or less, the presence of the sperm in the ampulla of the tube at the time of ovulation is essential for fertilization to occur. Sperms that are deposited in the uterine cavity are transported rapidly to the fallopian tubes and are discharged in the peritoneal cavity, where they are probably phagocytized. Thus to be successful, IUI needs to be closely timed with ovulation, or the alternatively repeated IUI may have to be performed in a given cycle [17]. On the other hand, the problem with IUI is that there is a risk of ascending infection when introducing the catheter into the uterine cavity [18]. There are also, some difficulties in introducing the catheter which may need excessive manipulation of the cervix, this may result in prostaglandin release or other hormones that may decrease fecundity or even some time lead to anaphylaxis [19]. Using washing technique with motility stimulant 'pentoxifylline' for IUI and ICI give better results because the washing techniques are known to improve the fertilization rate and then pregnancy rate by removing the deleterious substances from the seminal plasma [20], but PF can add more to the fertilizing potential of sperm and may further improve the clinical results of the artificial insemination.

In this study, the pregnancy rate per cycle of intrauterine insemination is 16.7%. This relatively high pregnancy rate pinpoints directly to the use of PF in the technique of sperm preparation, while other possibilities like a careful selection of the patients seems to be crucial in trying to achieve success with IUI because pure andrological indications are shown to be the best, followed cervical factor and then idiopathic infertility [21]. Moreover, the good follow up for the couples, the appropriate timing of the insemination and the use of gonadotropin in controlled ovarian stimulation can lead to increase in the pregnancy rate [22]. It has been reported that the combination of IUI with gonadotropin stimulation provides better results than clomiphene and IUI. The effectiveness of gonadotrophin treatment combined with IUI has been repeatedly demonstrated for a variety of infertility disorders, including endometriosis, cervical factor, and unexplained infertility. As with clomiphene, few pregnancies occur after 4 cycles of treatment [23]. The pregnancy rate per cycle with gonadotropin and IUI was 19% and 14.3% compared to 4% and 7.7% with clomiphene and IUI respectively [24]. In a Meta analysis of randomized trials comparing gonadotropin stimulation with IUI or with timed intercourse, IUI was associated with approximately a 2-fold greater pregnancy rate [25]. In a randomized trial, superovulation and IUI resulted in a 3- fold greater rate compared with intra-cervical insemination and 2 fold greater compared with either IUI alone or a combination of super ovulation and intra-cervical insemination [26]. In this study, the results of Intracervical insemination revealed that the pregnancy rate is 10.4%. This percentage seemed also higher than that of other studies. In a randomized trials review by O'Brien and Vandekerckhove [27] found that pregnancy rate per cycle of cervical insemination was 5%. The same results were obtained by Carroll and Palmer [28]. In both studies, the ICI was done by frozen donor sperms. Our relatively good pregnancy rate may be due to the use of PF in the sperm activation technique in vitro. Incubation of washed sperm with pentoxifylline may accelerate the capacitation process through biochemical changes in the cervical mucus. Fekil *et al.* [29] observed that the interaction between sperm and cervical mucus *in vivo* leads to biochemical changes, as 45 % decrease of the sperm cholesterol level and other lipid contents which affect positively on sperm capacitation. Although the statistical difference between IUI and ICI methods did not reach the significant level (Table 4-5),

The study suggests that IUI offers a greater likelihood for pregnancy than ICI in patients with poor semen quality and idiopathic infertility. This observation is consistent with other studies. Different studies compared between the pregnancy rate of IUI and ICI, some found that IUI is more effective than ICI when a frozen donor sperm is used [27, 30, 31]. In a review of randomized trials comparing IUI and ICI using fresh or cryopreserved semen [27], it has been found that IUI is more beneficial than cervical insemination when a cryopreserved donor sperm was used, however there was no difference in pregnancy rate between IUI and ICI when a fresh donor sperm was used.

CONCLUSION:

Sperm activation with pentoxifylline improves pregnancy rate of both Intrauterine and Intracervical Inseminations when managing unexplained or mild male factor infertility.

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