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Study the effect of piroxicam treatment for intrauterine insemination using density gradient for sperm preparation

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Abstract---Back ground: Endometrium contraction following intra-uterine insemination (IUI) was considered one of implantation failure causes. Aim of the study: The primary purpose of the study was to ascertain the effect of piroxicam treatment on pregnancy outcome following six days of intrauterine insemination. Materials and Methods: The study was accomplished in the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University through the period from Nov. 2020 till Oct. 2021. Forty infertile couples were selected randomly for IUI and divided into 20 couple treated with piroxicam and other 20 couples were not treated with piroxicam. The semen samples were prepared by discontinuous density gradient. Results: There was no significant differences regarding mean FSH level ($p=0.769$), LH level($p=0.79$) prolactin level ($p=0.299$), E2 level ($p=0.348$) and TSH level ($p=0.950$) between the studied groups. There was no significant difference in dominant follicles counts, follicular sizes or endothelial thickness between the two groups. The results showed a significant improvement in sperm motility and morphology ($p < 0.05$) after in vitro activation by density gradient technique compared to before activation. In the piroxicam group ,4 out of 20 women became pregnant while in non-piroxicam group ,3 spouses were pregnant 15%when density gradient activation technique was used. Conclusion: Although, IUI outcome was shown no significant different following the

treatment with and without piroxicam, it seemed that piroxicam can enhance the endometrium to improve the implantation process.

Keywords---piroxicam, intra-uterine insemination, density gradient technique.

Introduction

Infertility affects around 10%–15% of couples and is a significant component of therapeutic treatment. The National Center for Health Statistics' National Survey of Family Growth has shed light on the incidence of infertility in the United States. Findings include that 12.3% of married women reported having impaired fertility (Cheng *et al.*, 2020).

Female infertility may be classified into three major categories: abnormal ovulation, transport, and implantation. Hypothalamic and pituitary gland dysfunction may result in an excess of prolactin, which can impede ovulation. Infertile women are six to eight times as likely to develop endometriosis. Female infertility may be caused by gonorrhoea, peritonitis, past tubal surgery, and fimbrial adhesions; as a consequence, the egg is not released or stuck, hence delaying conception (Anwar & Anwar 2016)

The causative of infertility may be recovered by medicine or assisted reproductive technologies (ART). One of these techniques is the intra-uterine insemination. This method is a low- to moderate-complexity procedure performed before IVF programs (Ferber *et al.*, 2020). In a major randomized controlled experiment, it was shown that among infertile couples IUI significantly increased the probability of conception (odds ratio 3.6). In IUI, the women treated with aromatase inhibitors such as Letrozole which have been employed as an essential option in ovulation induction regimens. The drug inhibits the aromatization of androgens into estrogens, freeing the hypothalamic-pituitary axis from estrogen negative feedback (Russell and Grossmann, 2019).

The uterus is mostly made of smooth muscle that contracts and relaxes spontaneously and rhythmically (Nourel-Deen, *et al.* 2013). Outside of pregnancy, uterine contractions are responsible for the ejection of menstrual debris during menstruation. Embryo implantation is the most significant element limiting in-vitro fertilization (IVF) and embryo transfer. The interaction between embryo and endometrium is highly dependent on the quality of each. Due to the similarities of menstruation and implantation, it is beneficial to be familiar with other early indicators of pregnancy (Lama, 2017)

Piroxicam is a nonsteroidal anti-inflammatory drug (NSAID) that has been used prior to embryo transfer in a variety of studies with varying results. It inhibits the conversion of arachidonic acid to prostaglandin precursors at the rate-limiting cyclooxygenase enzyme step. The mechanism of action is similar to other NSAIDs, it is not totally known (Kothencz, 2020). Prostaglandin inhibitors or sedatives have been used to reduce or prevent uterine contractions or cramps prior to transfer. Similar to the current research, it has been demonstrated that piroxicam

treatment group had significantly higher rates of implantation and clinical pregnancy (Nyachieo et al. 2019). Thus, the objective of present study is to improve the IUI outcome using the piroxicam as a treatment to decrease the endometrium contraction negative effects.

Subjects and Methods

This is a prospective (comparative) study conducted in the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Al-Nahrain University, Baghdad- Iraq during the period between November 2020 and October 2021. The study included 40 infertile couples who attended the ART unit of the Institute and had been scheduled for IUI due to either primary or secondary infertility caused by various etiologies. Some of these infertile females were having their first assisted reproductive technique (ART) attempt, while others had already undergone at least one IUI cycle before. Ethical approval of the current study was issued by the Local Medical Ethical Committee of the "High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University". All of the patients were counseled, and written informed consents from them and after their agreement had been attained, they were within the inclusion criteria.

Patients

The infertile couples attending the infertility clinic were assessed thoroughly by full history-taking, physical examination, gynecological examination, evaluation of tubal patency (hysterosalpingography, or laparoscopy), and trans-vaginal ultrasound. Semen analysis was performed according to WHO 2010 and 2021.

Study Groups

The 40 infertile couples were selected randomly and divided into two groups as the following:

Group A: Consisting of (20) infertile couples the females received standard ovarian stimulation (Letrazole and/or gonadotropins) and underwent IUI with taking piroxicam after 6days of insemination.

Group B: Consisting of (20) infertile couples the females received standard ovarian stimulation (Letrazole and/or gonadotropins) and underwent IUI without taking piroxicam.

Pre-IUI workup

Pre-IUI work-up is recommended for all couples. In an ART clinic, female partners were evaluated by: Obtaining a complete history: The age of the female, the kind of infertility (primary or secondary), and the type of infertility (primary or secondary), infertile length, menstrual history, and any previous pregnancies were all taken into consideration. Thorough general examination, abdominal and pelvic examination to exclude any anomaly that may be a sign of the cause of infertility or may be a sign of the IUI or pregnancy are contraindications. Baseline hormonal evaluation was measured at early follicular phase (day 2 or 3 of menstrual cycle) namely : FSH, LH, and E2, as well as prolactin (PRL) and thyroid function tests TSH (thyroid stimulating hormone) and blood sugar levels that should have

been normal before commencing any ovarian stimulation and measuring another estradiol level on the day of the procedure ,using the Mini VIDAS Analyzer System.

Tubal Pathology Assessment: In previous cycles, this was done via hysterosalpingography, hysterosalpingo contrast sonography, or laparoscopy. Women with inadequate tubal patency or cavity deformity were excluded from the study. A vaginal scan was performed on the second or third day of the menstrual cycle to look for any pathology, such as an ovarian cyst, and to assess antral follicle numbers and/or the presence of PCO.

Ovulation Induction Protocols

The ovarian stimulation regimens chosen were -Letrozole pill 2.5 mg(Femara Novartispharma AG/Basilar Swiza) was taken twice daily for 5 days starting on day 3 of the cycle. HMG injections 75 IU of both urinary FSH and LH which were given intramuscularly, they were prescribed from day 5 of the cycle and the dose was adjusted according to the patient's response.

-Serial transvaginal ultrasound examinations to determine follicle number, size, and endometrial thickness were used to evaluate the follicles' maturation. To give the trigger, the dominant follicle should be 17-23 mm in size.-On day 12 or 13 and when at least one follicle reaches 17 mm, 6500 IU /vial (250mg) of recombinant human Chorionic Gonadotropin (hCG) was given subcutaneously. Females were prepared for IUI, which actually takes place 36-40 hours after recombinant hCG trigger.

Male Preparation for IUI

In this study male partner was assessed by andrology specialist in the male infertility clinic involving: History: Age, occupational status, developmental history, medical history, surgical operation, drugs and habits and sexual history. Examination: General and scrotal examination. Seminal fluid analysis (SFA): was done according to WHO laboratory manual for the Examination and processing of human semen 2010 and 2021. For those samples with mild male factor another SFA was done before joining stimulated- IUI cycle testing post-wash.

Density Gradient technique

This technique was done by adding one ml of 80% of Sil-Select Plus gradient(Ferticult[®] , Belgaum) as a first layer solution in a test tube followed by one ml of 40% of Sil-Select Plus gradient(Ferticult[®] , Belgaum) as a second layer solution, and then liquefied semen sample was added on the second layer. This test tube was carefully put in a centrifuge at 3000 rpm for 15 minutes. Then the supernatant was discarded and one ml of Ferticult Flushing medium(Ferticult[®] , Belgaum) was added to the pellet and put in an air incubator for 30 minutes at 37°C. A drop of 10 µl was aspirated and put on a slide with coverslip and examined under the microscope at 400X magnification power to assess the certain sperm function parameters(Hindal, A.S., Mossa, H.A. and Abood, M.S., 2018.)

Intrauterine Insemination Protocol (IUI)

The intrauterine insemination technique was performed in an ordinary gynecological room with a clean sterile technique. After preparing the sample, activated and processed sperms were aspirated in a syringe pointed to (0.5ml) and then this syringe attached to IUI catheter. The female partner was prepared for the insemination process by lying down in a lithotomy position on the couch with draping, a warmed and lubricated Cusco speculum was introduced gently inside the vagina to visualize the uterine cervix and slightly down word to expose the cervix, which was washed with saline. The cervical mucus was removed by a small piece of cotton and the cervix was prepared by using aseptic techniques (Merviel et al., 2010). The catheter was then gently passed through the cervical canal which is a very thin flexible rubber tube and by using a marker guideline for the correct placement, the washed semen sample was pushed slowly and the catheter then removed gently and gradually . Patient was instructed to spin on the side of the ovulating ovary for (30) minutes then resumed normal activity.

Piroxicam treatment

Piroxicam (Kontam pharmaceuticals,china) suppositories 20 mg single dose is given 6 days after day 0 of IUI procedure rectally at any time of day 6.

Statistical analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 .The descriptive statistics including frequency, mean, standard deviation or error were measured to describe the data .The groups were compared by applying analysis of variance (ANOVA) between more than two groups then least significant test , paired samples t-test (before and after activation), repeated measures ANOVA (before and after activations in different groups), independent sample t-test (unpaired t-test between two groups) and chi square for non continuous data or percentage. The results were considered statistically significant when p value was less than 0.05.

Results

Comparison of demographic features between the studied groups

The mean age, body mass index and duration of infertility were expressed in mean plus minus standard error while the type of infertility expressed in frequencies. According to the results there was no significant difference in mean patients age between the studied groups. Moreover, no significant difference was recorded in mean body mass index, duration of infertility and type of infertility between the four groups as demonstrated in table 1.

Table 1: Demographic features of the studied groups

Parameter	Piroxicam +DG group	Control +DG group	P Value

	(Mean ± SE)	(Mean ± SE)	
Age (years)	29.5 ± 1.28	32.15 ± 2.01	0.093 ¥ NS
BMI (kg/m ²)	29.89 ± 1.08	28.97 ± 1.21	0.488 ¥ NS
Duration of infertility (years)	6.93 ± 1.50	5.73 ± 0.86	0.078 ¥ NS
Type of infertility no.(%)	Primary 9 (45%) Secondary 11(55%)	Primary 13(65%) Secondary 7(35%)	0.289 Ĉ NS

SE: Standard error; DG: Density gradient; SL: Simple layer; BMI: Body mass index; NS: Not significant ($p \geq 0.05$) ;¥: ANOVA test ;Ĉ:Chi square test.

Comparison of the hormonal levels between the studied groups

Comparison of hormonal levels between the studied groups were also expressed in mean plus minus standard error that illustrated in table-2. There was no significant differences regarding mean FSH level ($p=0.769$), LH level ($p=0.072$) prolactin level ($p=0.099$), E₂ level ($p=0.148$) and TSH level ($p=0.950$).

Table 2: Comparison of hormonal levels between the studied groups

Reproductive hormonal profile	Piroxicam+DG group (Mean ± SE)	Control+DG group (Mean ± SE)	P Value
FSH(mIU/ml)	7.56 ± 0.39	7.64 ± 0.47	0.769 ¥ NS
LH (mIU/ml)	7.66 ± 0.56	8.14 ± 0.60	0.072 ¥ NS
Prolactin (mIU/ml)	19.81 ± 1.12	21.10 ± 1.52	0.099 ¥ NS
E ₂ (pg/ ml)	157 ± 8	148 ± 11	0.148 ¥ NS
TSH (mIU/ml)	2.39 ± 0.23	2.56 ± 0.23	0.950 ¥ NS

SE: Standard error ; FSH: Follicle stimulating hormone ;LH : Luteinizing hormone;E₂: Estradiol ;TSH : Thyroid stimulating hormone; DG: density gradient NS: Not significant ($p \geq 0.05$) ;¥: ANOVA test.

Comparison of sonographic features between the studied groups

The comparison of endometrial thickness follicular counts, and follicular sizes between the studied groups were demonstrated in table -3, accordingly there was no significant difference in dominant follicles counts ($p=0.055$), dominant follicles sizes ($p=0.519$) and endometrial thickness ($p=0.894$) between the four studied groups.

Table 3: Comparison of sonographic features between the studied groups

Sonographic features	Piroxicam+DG group (Mean \pm SE)	Control+DG group (Mean \pm SE)	<i>p</i> value
Dominant follicles count	1.35 \pm 0.13	1.7 \pm 0.22	0.055 ¥ NS
Dominant follicles size	20.73 \pm 0.43	19.39 \pm 0.45	0.519 ¥ NS
Endometrial thickness (mm)	8.73 \pm 0.21	8.66 \pm 0.27	0.894 ¥ NS

SE: Standard error ; DG: Density gradient; SL :Simple layer ; NS: Not significant ($p \geq 0.05$) ;¥: ANOVA test.

Comparison of seminal fluid analysis parameters before and after activation technique

In vitro preparation and activation of semen by density gradient before and after activation were compared by application of paired sample t test. The results showed significant improvement in sperm motility and morphology ($p < 0.05$) in density gradient technique; however there was no significant decline in sperms concentration after activation with p value equal to 0.894 as illustrated in table 4.

Table -4: Comparison of SFA parameters before and following density gradient technique

Certain Sperm Function Parameters	Before activation (Mean \pm SE)	DG (Mean \pm SE)	After DG activation (Mean \pm SE)	<i>p</i> value
Sperm concentration ($\times 10^6$ /ml)	58.23 \pm 3.93		55.19 \pm 6.13	0.513 ¥ NS
Progressive motile sperm %	33.15 \pm 2.16		84.3 \pm 3.44	< 0.001 ¥ S
Non progressive motile sperm %	36.00 \pm 1.16		11.1 \pm 3.10	< 0.001 ¥ S
Immotile sperm %	30.37 \pm 3.87		4.60 \pm 2.24	< 0.001 ¥ S
Morphologically normal	33.12 \pm 1.20		62.70 \pm 2.10	< 0.001 ¥ S

sperm %			
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DG: Density gradient; SE: Standard error NS: Not significant ($p \geq 0.05$) ;S: Significant ($p < 0.05$) ; T: Paired sample t test.

Pregnancy rate of the studied groups

In piroxicam group 4 females out of 20 women became pregnant while in control group 3 spouses became pregnant, however there was no significant difference in pregnancy rate between the two groups of patients ($p=0.135$) as presented in table -5

Table -5: Pregnancy rate of the studied groups

Study Groups	Negative pregnancy N. (%)	Positive pregnancy N.(%)	p value
Piroxicam with density gradient	16 (80%)	4 (20%)	0.677 Ĉ NS
Control with density gradient	17 (85%)	3 (15%)	

NS: Not significant ($p \geq 0.05$) ;Ĉ:Chi square test.

Discussion

Results of present study found no significant differences in demographic features namely; Age, BMI, type and duration of infertility between the two groups. There was no significant difference among the BMI of females treated with piroxicam and those not treated. This finding indicated that the two groups had comparable age which is important to eliminate any variable that may affect the results of the study. Data of current study showed no significant difference between the levels of FSH, LH, prolactin and Estradiol measured in the menstruation day 2-3 of ovulation induction. FSH stimulates the development of ovarian follicles in granulosa cells, whereas LH action is involved in follicle development and maturation (Sudhakaran et al.,2022)

Endometrial thickness has been explored as a predictor of pregnancy outcome (Al-Dujaily and Aburgheef, 2012). It has been recorded that, with increasing endometrium thickness, up to 14 mm, the pregnancy rates will decline. The data of current work indicate that sperm preparation technique used to increase fertilization rates. There was a significant difference between the results of ART treatment (French et al. 2010) which obtained a pregnancy rate per cycle of 12.5% with less than 10 million spermatozoa/mL and of 17% with more than 20 million.

The technique used for in vitro activation in the current study is the discontinuous density gradient technique which gave the best results in sperm active sperm motility and morphologically normal sperm percentage. Semen preparation may modify sperm characteristics considerably, and the number of

motile sperm and the morphological criteria should logically be assessed after semen preparation(Mahajan,2014)

Pregnancy rate of the studied groups

Results of a prospective study showed significant increase in clinical pregnancy rate (CPR), in patients treated with piroxicam in relation to the control group. There was no significant difference in pregnancy rate between the groups of patients. The effect of this medicine to prevent the prostaglandin (PG) action may be related to the uterine contraction. The primary goal of research in the field of assisted reproduction is to increase pregnancy rates. The initiation of uterine contractions can result in the embryos being expelled immediately or later, which has been a real concern in the other groups that not used piroxicam (Mansour, 2012). Consequently, the present study concluded that IUI outcome did not reveal a significant differences by piroxicam treatment, however ,it improve the pregnancy rate. More studies are recommended by increasing the sample size and piroxicam dose.

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