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EFFECT OF INTRAUTERINE FLUSHING OF HCG ON DAY OF TRIGGER ON IUI OUTCOME

Al-Tamemi K. I. A. [1], Abood M. S. [2], Al-Ammar M A [2]

[1] Specialist in obstetrics and gynecology

[2] High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq

*Kinanaalwan@gmail.com

Abstract

Intrauterine insemination (IUI) has been considered as common in infertility treatment. It is commonly used in couples with mild male factor infertility, unexplained infertility, mild endometriosis, cervical factors and psychological sexual dysfunction. Implantation has been considered as a crucial phase of pregnancy. It includes a multifaceted process because more than half of all pregnancies become failures mainly due to the cause of implantation failure. In the process, there are three most important components for the successful implantation, which includes process-embryo quality, endometrial receptivity, and embryo–endometrium communication. Aim of study: To evaluate the effect of intrauterine flushing of 1000IU hCG on day of trigger on pregnancy rate in infertile females undergoing IUI. Material and method: The present study was applied on 80 infertile females attending the High Institute for Infertility Diagnosis and ART/ Al- Nahrain university during the period from November 2020 to May 2021. These females were subjected to ovarian stimulation protocol using letrozole and hMG in doses adjusted according to their response. These females were scheduled to undergo IUI in the infertility clinic of the institute. Ultrasound monitoring was done for follicular maturity and when the leading follicle reach 17-19 mm triggering was done using subcutaneous hCG and on that day hCG 1000 IU was flushed gently using IUI catheter for 40 females (study group) while the control group did not have any intervention. Both groups were subjected to IUI 36-38 hour after hCG trigger preceded by ultrasound assessment to make sure of ovulation. Results: In the present study, it has been found that there are no significant differences regarding initial characteristics, indication for IUI, basal cycle characteristics, ovarian stimulation characteristics and semen parameters before and after processing while there was a substantial difference among both groups regarding biochemical and clinical pregnancy rates. Conclusion: In IUI cycles, intrauterine human chorionic gonadotropin (hCG) flushing done on the day of hCG activation can improve biochemical and clinical pregnancy rates.

Key words: IUI, hCG flushing, pregnancy rate, infertility.

Introduction

One of the most common infertility treatments is known as Intrauterine insemination (IUI). Mohammadi *et al*. (1) mentioned that it is commonly used in the couples with unexplained infertility, mild endometriosis, cervical factors and mild male factor infertility. In most countries, IVF/ICSI registries regardless of the lack of intrauterine insemination (IUI) registering or combination of IUI procedures has been reported. With respect to this, it is evident that IUI for different causes may be considered as important means of assisted reproduction across the world (2). IUI has been also called as artificial insemination (AI), which is the process by which fresh or frozen sperms are placed into the female reproductive tract after separation of seminal plasma via means other than sexual intercourse (3).

Intrauterine insemination has been directed for a wide variety of situations with or without ovarian stimulation, whereas the most crucial indication includes male infertility, particularly in the circumstance when sperm donation is required. According to the Bensdorp *et al*. (4), it is also pointed out for couple's complaints of minimal, mild endometriosis and all types of unexplained infertility. IUI might be reflected in stimulated cycles at the time of waiting for IVF/ICSI program. In addition, it may also be considered when IVF/ICSI is not advised in women with patent fallopian tubes. In the majority of these indications, natural IUI or stimulated IUI cycles is an empiric management. Since, it is probably that most infertility causes involve untreatable or unknown factors (5).

The study by Eccles (6) defined endometrial receptivity as the temporary unique sequence of aspects which become resultant of endometrium receptive to the embryonic implantation. Receptive endometrium is one of the cornerstones for the embryo successful implantation, and it's appropriately primed to implant the embryo. The development of receptive endometrium has been controlled through various growth factors, steroid hormones and cytokines. Adequate blood supply of the endometrium is obligatory for these factors to be available in the endometrium (7).

hCG has been considered as the heterodimeric hormone. Earlier to the implantation, hCG has been secreted through the blastocyst. hCG's primary role through the corpus luteum is to stimulate progesterone production. Therefore, its role is to modify the maintenance of the upcoming pregnancy, which is primarily by ratifying uterine angiogenesis. Additionally, Simopoulou et al. (8) mentioned that it assures the maternal tolerance of the semi-allograft embryo. It also assists the uterine enlargement that goes around fetal development.

As per the study of Santibañez et al. (9), it has been found that hCG primarily controls the endothelial cell in order to respond to interleukin 1. Additionally, it also intensifies the cytokine-mediated impact on the release of angiogenic factors, migration and cell proliferation. At the maternal-fetal interface, widespread angiogenesis is required for embryo implantation. With respect to this, at the time of implantation window, hCG helps in modulating the receptivity of the endometrial stromal cells to interleukin-1 through upregulating its receptor (IL1R), which has an influence on angiogenesis that is a pathway for the promotion of embryonic growth (9).

hCG also supports trophoblast apposition. At the first stage of implantation, it helps in losing the alignment of the trophoblast to the decidua, whereas at the second stage of implantation, which is adhesion, it supports the trophoblast's closer supplement to the decidua. Additionally, it also supports the endometrium through modifying proteins included in implantation (10).

Licht et al (11) studied the influence of hCG on the human endometrium. The study experiment revealed that intrauterine 500 IU of hCG/mL provoked an important inhibition of

intrauterine insulin-like growth factor-binding protein 1 (IGFBP-1) and macrophage colony-stimulating factor (M-CSF). In contrast, matrix metalloproteinase 9 (MMP-9), vascular endothelial growth factor (VEGF) and leukaemia inhibitory factor (LIF) were significantly stimulated (12).

Methods

During the time period between November 2020 and May 2021 in the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al- Nahrain University, Baghdad, Iraq this prospective case control study was carried out.

The study included 80 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 attempt, while others had already undergone at least one IUI cycle before.

All infertile couples were subjected to full history taking, complete general and gynecological examination and full infertility investigations including husband's seminal fluid analysis, hormonal assay at early follicular phase, trans-vaginal ultrasound and hysterosalpingography for evaluation of uterine cavity and tubal patency and/or laparoscopy for assessment of tubal patency and exclusion of pelvic pathology.

- Ethical approval of the current study was issued by the Local Medical Ethical Committee of the "High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University". In addition, written informed consent was obtained from each participant included in the study. From all of the participants in this study, written informed consents were obtained prior to the research. After their agreement had been attained, they were within the inclusion criteria. All patients enrolled in the study fulfilled the following criteria: Age: 18-40 years old females with patent both or one fallopian tube, the BMI was between 18-40 kg/m^2. Male partners with normal or subnormal semen analysis and should have a normal thyroid function test.
- The 80 infertile females were selected randomly and divided into three groups:
  - **Group A:** The control group consisted of (40) infertile females, who received standard ovarian stimulation (letrozole and/or gonadotropins) and underwent IUI without intrauterine human Chorionic Gonadotropin flushing.
  - **Group B:** The study group and consisted of (40) infertile females, they received standard ovarian stimulation (letrozole and/or gonadotropins) and underwent IUI with intrauterine human Chorionic Gonadotropin flushing 1000 IU done on the day of hCG trigger.

All couples should undergo pre-IUI workup. Female partners were assessed in ART clinic by complete history, **t**horough general examination, abdominal and pelvic examination to exclude any contraindication to IUI or pregnancy and hormonal analysis by an enzyme-linked fluorescent assay (ELFA) technique for baseline hormonal evaluation at early follicular phase (day 2 or 3 of the menstrual cycle): FSH, LH, and E_2 in addition to an assessment of PRL, thyroid-stimulating hormone (TSH) which should be normal before starting any ovarian stimulation and another estradiol level measured in the day of ovulation triggering. Tests for Human Immunodeficiency Syndrome (HIV) and Hepatitis viruses were done for both partners. All females should have one or both tubes diagnosed by hysterosalpingography, laparoscopy or Saline-Infusion Sonography. TVUS was done to exclude ovarian cyst and uterine anomalies.

The Body Mass Index was estimated by distributing the weight in kilograms on the height in square meters (kg/m^2).

From day three of the cycle for five days, letrozole tablet 2.5 mg was given two times daily. Recombinant FSH 75 IU / vial which was given subcutaneous or hMG injections 75 IU of both urinary FSH and LH, it was given intramuscularly from day 5 of the cycle and the dose was adjusted according to patient's response. The maturity of the follicles was monitored by serial transvaginal ultrasound examinations to evaluate follicle number, size and endometrial thickness. The size of the dominant follicle should be between 17-23 mm

to give the trigger. On day 12 or 13 and while as a minimum one follicle extends 17 mm, 6500IU /vial(250mg) of recombinant human chorionic gonadotropin (hCG) was given subcutaneously.

hCG consists of adding 1000 IU (0.4ml) of hCG to 0.1 mL of normal saline in the preparation of the intrauterine injection (IJ). In the present study in order to attain a dose of 1000 IU of hCG, dilution was created with the lowest level of sterile saline. Uterine flushing by hCG was performed for group B. The intrauterine human Chorionic Gonadotropin flushing was done on the day of hCG trigger in group B. In case of pelvic pain, women were asked to take paracetamol if needed and avoid nonsteroidal anti-inflammatory drugs. After complete infusion the catheter was withdrawn

The women in the group (B) at the day of hCG trigger were asked to lie in the lithotomy position and the cervix was visualized with a vaginal speculum, Firstly, removal of excess vaginal and cervical secretions with a sterile piece of gauze was done, then an intrauterine insemination cannula was presented into the uterine cavity by the cervical canal, preferably without the use of a vulsellum. Then, 0.5 mL of hCG that contains 1000 IU hCG (hCG vial 5000 IU was diluted by 2.5 sterile saline so 0.5 of the solution contains 1000 IU hCG) was injected intrauterine through the cannula, no premedication was given. Females were prepared for IUI which was done 36-40 hours' post triggering by recombinant hCG. Post IUI luteal phase support was given in the form of progesterone suppositories to all patients for fourteen days.

If the patient who had no menstruation, after the fourteen days of IUI, pregnancy was detected through using serum β-hCG test. After two weeks, in order to confirm the existence of an intrauterine gestational sac, a vaginal ultrasound was executed. The results of the tests were recorded for analysis.

With the help of Microsoft Excel version 2016 and SPSS for Windows version 21, the data gathered in the study were statistically analyzed. The significance level was set at 0.05. Analysis of difference between the three groups was performed using one-way ANOVA When a significant difference was found in chi-squared test results, a group-to-group analysis was performed using either chi-squared test or Fisher's Exact test, setting the significance level at 0.017, according to Bonferroni's correction.

Results

1- Demographic characteristics of all infertile females enrolled in the present study

The demographic characteristics of infertile women enrolled in the current study are shown in Table (1). The total number of cases was 80 infertile women with different causes of infertility who were divided randomly into two groups, each group was 40 infertile women (n=40).

The age was in the range of 20 – 40 years old and a mean of 30.65 ± 5.17 years for group A, and a mean age of 30.93 ± 5.30 years for group B. The mean body mass index was in the range of 18.4 – 39.1 kg/m^2 and a mean of 28.53 ± 4.99 for group A, in the range of 21.7 – 37.1 kg/m^2 with a mean of 29.56 ± 3.88 for group B.

The parity was in the range of 0 – 5 for the group (A) and in the range of 0-4 for the group (B) with a median of 0 for both groups. The number of previous miscarriages was in the range of 0-3 for group A, 0-2 for group B with a median of 0 for both groups. The type of infertility was described as that 42% of women had primary infertility while 57.5% of them had secondary infertility in group A, while; 55% of women had primary infertility while 45% of them had secondary infertility in group B.

The duration of infertility was in the range of 1-14 years and a median of 5 years in group A, but it was in the range of 2-17 years and a median of 6 for group B. The number of previous IUI cycles was in the range of 1-3 years for group A and a median of 0 for both groups.

There were no significant differences between women of the two groups regarding age, BMI, parity, no. of previous miscarriages, type and duration of infertility or number of previous IUI cycles (table- 1).

2- Difference between Groups regarding Indication for IUI:

Indications of IUI included male factors, anovulation (PCOS), unilateral tubal block, mild endometriosis, unexplained infertility and combined male and female Factors. For group A, the indications of IUI were 27.0% for male factors, 32.5% for anovulation (PCOS), 7.5% for unilateral tubal block, 2.5% for mild endometriosis, 17.5 for unexplained infertility and 12.5% for combined male and female Factors.

For group B, the indications of IUI were 25% for male factors, 37.5% for anovulation (PCOS), 7.5% for unilateral tubal block, 0% for mild endometriosis, 17.5% for unexplained infertility and 12.5% for combined male and female Factors. (table 2).

3- Difference between groups regarding basal cycle characteristics

On the early follicular phase, cycle regularity and hormonal analysis comparison for all females enrolled in the present study was done (cycle day 2 or 3 of the cycle) in Table 3. The menstrual cycle was regular in 65% of women in group A and in 55% of women of group B. The basal FSH in IU/L was in the range of 2.4 – 10.1 IU/L and a median of 7.6 in group A, in a range of 2.4 – 10.1 IU/L and a median of 6.9 in group B.

The basal LH in IU/L was in the range of 1.8 – 11.9 IU/L and a median of 6.7 in group A, in a range of 1.8 – 14.8 IU/L and a median of 6.2 in group B. The basal estradiol level in (ng/ml) was in the range of 13.4 – 51.0 ng/ml and a median of 43 in group A, in a range of 5 – 45.1 IU/L and a median of 32 in group B. There were no significant differences between women of the two groups regarding menstrual regularity, or basal levels of serum FSH, LH, or estradiol (table 3).

4- Difference between groups regarding ovarian stimulation characteristics:

The ovarian stimulation drugs used in the current study were either letrozole, hMG, u-FSH, letrozole + hMG or letrozole + u-FSH. In both groups, letrozole was used in fifty percent or more of the cases.

In group A, letrozole was used in 60% of women, hMG in 12.5%, u-FSH in 2.5%, letrozole + hMG in 20% and letrozole + u-FSH in 5% of women. In group B, letrozole was used in 65% of women, hMG in 12.5%, u-FSH in 0%, letrozole + hMG in 7.5% and letrozole + u-FSH in 15% of women. The number of mature follicles was in the range of 1-4 and 1-3 for groups A, B respectively with a median of 2 for both groups. The maximum dimension of the dominant follicle in (mm) was 18-24 mm for both groups (A and B) with a median of 20mm for both groups.

The midcycle estradiol levels in (ng/ml) were highest in group B. The midcycle estradiol levels were in the range of 108 – 603 ng/ml and in a median of 284ng/ml in women enrolled in group A, in the range of 80 – 908 ng/ml and in a median of 301ng/ml in women enrolled in group B. The peak endometrial thickness in(mm) was in the range of 8.1 – 11.5mm and a median of 9, in a range of 8.2 – 10.2mm and a median of 9.2 in groups A, B respectively.

There were no significant differences between women of the two groups regarding ovarian stimulation drugs number of mature follicles, maximum dimension of the dominant follicle, midcycle serum estradiol level or peak endometrial thickness (table 4).

5- Difference between Groups regarding Pregnancy Outcomes:

Both Biochemical and clinical Pregnancy rates were the highest in group B, the biochemical pregnancy rate was 7.5%, 40% while the clinical pregnancy rate was 5%, 40% in group A and B respectively, multiple pregnancies only had been seen in group B (2.5%) and ectopic pregnancy was 0% in both groups (table 5).

Discussion

Implantation has been considered a crucial phase of pregnancy. It includes a multifaceted process because more than half of all pregnancies become failures, mainly due to the cause of implantation failure. In the process, there are three most important components for successful implantation, which includes embryo–endometrium communication, endometrial receptivity and process-embryo quality (13).

Moreover, in order to improve the results of assisted reproduction treatments around the time of ET, IUI administration of hCG has the capacity, however, nonrandomized trials and randomized trials showed varying results (14).

After the ovulation induction, the influence of the maternal age on IUI outcomes has been studied in a number of researches, which revealed that diminished pregnancy rates in patients more than 40 years as compared to women less than 40 years across various infertility etiologies, including male factor, unexplained infertility and mild endometriosis (15). On the other hand; the reproductive risk of infertility increased mainly due to obesity because of menstrual dysfunction and oligo-anovulation (15). The type of infertility, the period of infertility and incidence of previous trials of IUI all these parameters were analyzed in the present study and data showed no significant difference between groups. This random allocation and the lack of significant difference among enrolled infertile women is crucial to avoid any bias in the primary and secondary outcomes.

IUI is indicated in cases of unexplained infertility, male subfertility, unilateral tubal blockage, cervical or ovulatory dysfunctions, and mild or minimal endometriosis (16). A comparison was done between the indication of IUI in the two groups of infertile females. The statistical analysis revealed no significant difference in the percentage of different infertility causes between the tested groups providing scientific avoidance of any bias in the IUI outcome.

The development of ovarian follicles has been stimulated by FSH in the granulosa cells, whereas in follicle maturation and development, LH action is involved. Therefore, female fertility reduces due to deficiency in FSH and LH production and action, which are in screening for declined ovarian reserve (17).

On the third day of a spontaneous menstrual cycle, the measurement of FSH and LH has been linked with the AMH and E2 measurement. Besides it is vital in order to estimate ovarian reserve to personalize ovarian stimulation protocol particularly in underdoing ART procedures in women (18). In screening for declined ovarian reserve, an amalgamation of E2 and FSH appears to be more sensitive as compared to either test alone. Estradiol is a product of granulosa cells and can be considered a reflection of follicular activity (19).

The statistical analysis of the present study showed no significant difference in the level of basal hormones between females in both groups. This finding indicated that the two groups had comparable ovarian reserve which is important to eliminate any variable that may affect the results of hCG flush in IUI treatment.

The ovarian stimulation drugs used in the current study were either letrozole, hMG, r-FSH, letrozole + hMG or letrozole + r-FSH, in all groups, letrozole was used in about fifty percent or more of the cases. Using of letrozole may have a positive effect on the pregnancy rate as the previous study showed that mean of hyaluronic acid (HA) was elevated significantly in women who received letrozole plus FSH compared to women who received Clomiphene citrate plus FSH as it is known that in the formation of the cellular microenvironment, HA plays a crucial role, which is conducive to the development of the proliferative process of the endometrium (20).

There was no significant difference among tested groups in regard to drugs used for achieving ovulation induction, if there was a significant difference, it may affect the IUI outcomes.

With respect to preovulatory follicles, in the IUI outcome, a good prognostic predictor includes the number of follicles. In cycles with three preovulatory follicles (>15mm), the highest pregnancy rate has been found. Multifollicular development leads to a rise in the number of fertilized oocytes. In addition, it also leads to a better quality of endometrium and luteal phase. In this manner, it improves fertilization and pregnancy rates (21).

The biochemical and clinical pregnancy rates were significantly higher in women of group B when compared to women of group A.

The present study is the second one that exhibited the effect of hCG injection on the day of

ovulation trigger after Fahmy, et. al., (22). In order to investigate the effects of intrauterine hCG injection on the outcome of the IUI cycles, in this study a randomized clinical trial was used. Prior to the IUI, at different timings in the same doses, the intrauterine hCG was administered. The results indicate that the injection of 1000 IU on the day of the trigger would improve the outcomes.

Nonetheless, in the research, a difference has been found involving intra-uterine hCG administration prior to either blastocyst transfer or past embryo transfer. Additionally, Makrigiannakis et al. (23) stated that hCG Types has been employed either from isolation from urine or was created with the help of technology like recombinant technologies. In addition to this, hCG was also used in different time points and concentrations earlier to embryo transfer.

The study by Berndt et al. (24) pointed out that the main step in placental formation is the display of strong angiogenic by hCG with the help of receptor stimulation of transforming growth factor beta (TGF-β) in endothelial cells (24, 25). The higher section hCG embryos in the IVF cycles show a positive correlation in their culture media with the implantation rate (26). In addition, the previous study demonstrated the correlation between hCG levels in the culture media and embryo quality and the results show a positive significant correlation in hCG level and a number of (Grade1 and Grade2) embryos (27).

Also several researchers as Gao et al., (28); Tan et al., (29) and very recently Asbagh et al., (30) found that infusion of hCG at different times and by different doses before embryo transfer improved the IVF/ICSI outcomes. During general anesthesia, after the oocyte retrieval, the intrauterine administration of 500 IU of hCG might efficiently raise the implantation and pregnancy rates (31).

In contrast, studies accomplished by Hou et al., (13) Osman et al., (32), Firouzabadi et al., (33) and Wire Leitner et al., (34) has reported dissimilar results and suggested that the use of intrauterine hCG injection before ET does not improve IVF/ICSI outcomes. Notably, after intrauterine hCG application, adverse influence on clinical pregnancy rates has been found which was principally true for patients without defined repeated implantation failure (RIF) (35).

In the present study and regarding group B, on the day of hCG, intrauterine hCG infusion was triggered as Fahmy and his colleagues did in their study in 2015(22). However, with different doses and smaller infusion volume, clinical and biochemical pregnancy rates were the highest in this group, it was a highly significant difference between group A (control group) and B (study group)

The explanation for these promising results is proposing that hCG molecules need more time to exert their best effect in the female genital tract. A number of researchers like Hong et al. (36) and Licht et al. (37) stated that more time is required by the drug in order to influence the endometrium mainly in ART cycles for the purpose of decreasing the uterine dyssynchrony and progression activated by ovarian stimulations. In addition to this, for the survival of the endometrial stroma, it encourages the appearance of important markers (38).

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Table-1: Difference between Groups regarding initial characteristics.

Characteristics	Group A Control Group (n=40)	Group B Study group (n=40)	*P- value*
Age (years) Range Mean ± SD	20 – 40 30.65 ± 5.17	21 – 39 30.93 ± 5.30	0.917 [1] NS
BMI (kg/m^2) Range Mean ± SD	18.4 – 39.1 28.53 ± 4.99	21.7 – 37.1 29.56 ± 3.88	0.342 [1] NS
Parity Range Median (IQR)	0 – 5 0 (0 – 2)	0 – 4 0 (0 – 1)	0.766 [2] NS
No. of Previous Miscarriages Range Median (IQR)	0 – 3 0 (0 – 1)	0 – 2 0 (0 – 0)	0.431 [2] NS
Type of Infertility Primary Secondary	17 (42.5%) 23(57.5%)	22 (55.0%) 18 (45.0%)	0.531 [3] NS
Duration of Infertility (years) Range Median (IQR)	1 – 14 5 (3 – 7)	2 – 17 6 (4 – 9)	0.183 [2] NS
No. of Previous IUI Cycles Range Median (IQR)	0 – 3 0 (0 – 2)	0 – 3 0 (0 – 1)	0.113 [2] NS

BMI body mass index – IUI intrauterine insemination
SD standard deviation – IQR interquartile range
Data presented as range, mean ± SD; range, median (IQR); or frequency (percentage)
1 Analysis using one-way ANOVA test
2 Analysis using Kruskal-Wallis test
3 Analysis using chi-squared test
NS non-significant

Table- 2: Difference between two Groups regarding Indication for IUI.

Indication for IUI	Group A Control Group (n=40)	Group B Study group (n=40)	*P- value*
Male Factor	11 (27.5%)	10 (25%)	
Anovulation	13 (32.5%)	15 (37.5%)	
Unilateral Tubal Block	3 (7.5%)	3 (7.5%)	0.586 [1]
Endometriosis	1 (2.5%)	0 (0%)	NS
Unexplained Infertility	7 (17.5%)	7 (17.5%)	
Combined Male and Female Factors	5 (12.5%)	5 (12.5%)	

IUI intrauterine insemination
Data presented as frequency (percentage)
1 Analysis using chi-squared test
NS non-significant

Table 3: Difference between Groups regarding Basal Cycle Characteristics hormonal analysis.

		Group A Control Group (n=40)	Group B Study group (n=40)	*P- value*
Menstrual Regularity	**Regular**	26 (65%)	22 (55%)	0.104 [1]
	Irregular	14 (35%)	18 (45%)	NS
Basal FSH (IU/L)				
Range		2.4 – 10.1	2.0 – 10.0	0.936 [2]
Median (IQR)		7.6 (5.3 – 9.3)	6.9 (6 – 8.9)	NS
Basal LH (IU/L)				
Range		1.8 – 11.9	1.8 – 14.8	0.365 [2]
Median (IQR)		6.7 (4.3 – 7.9)	6.2 (4.6 – 9.3)	NS
Basal Estradiol (ng/ml)				
Range		13.4 – 51.0	5.0 – 45.1	0.067 [2]
Median (IQR)		43 (41.6 – 43.8)	32 (25.1 – 40.0)	NS

IUI intrauterine insemination – IQR interquartile range
FSH follicle stimulating hormone – LH luteinizing hormone
Data presented as frequency (percentage); or range, median (IQR)
1 Analysis using chi-squared test
2 Analysis using Kruskal-Wallis test
NS non-significant

Table-4: Difference between Groups regarding Ovarian Stimulation Characteristics.

Ovarian Stimulation Drugs	Group A Control Group (n=40)	Group B Study group (n=40)	*P- value*
Letrozole hMG u-FSH Letrozole + hMG Letrozole + u-FSH	24 (60%) 5 (12.5%) 1 (2.5%) 8 (20%) 2 (5%)	26 (65%) 5 (12.5%) 0 (0%) 3 (7.5%) 6 (15%)	0.064 [1] NS
No. of Mature Follicles Range Median (IQR)	 1 – 4 2 (1 – 2)	 1 – 3 2 (1 – 2)	0.129 [2] NS
Max Dimension of Dominant Follicle (mm) Range Median (IQR)	 18 – 24 20 (19 – 21)	 18 – 24 20 (19 – 21)	0.471 [2] NS
Midcycle Estradiol (ng/ml) Range Median (IQR)	 108 – 603 248 (194 – 342)	 80 – 908 301 (218 – 505)	0.104 [2] NS
Peak Endometrial Thickness (mm) Range Median (IQR)	 8.1 – 11.5 9 (8.4 – 9.2)	 8.2 – 10.2 9.2 (8.3 – 9.3)	0.951 [2] NS

IUI intrauterine insemination – IQR interquartile range
hMG human menopausal gonadotropin – u-FSH urinary follicle stimulating hormone
Data presented as frequency (percentage); or range, median (IQR)
1 Analysis using the chi-squared test
2 Analysis using Kruskal-Wallis test
NS non-significant

Table 5: Difference between Groups regarding Pregnancy Outcomes.

	Group A Control Group (n=40)	Group B Study group (n=40)	*P* - value
Biochemical Pregnancy	3 (7.5%)	16 (40.0%)	0.003 S
Clinical Pregnancy	2 (5.0%)	16 (40.0%)	<0.001 (HS)
Multiple Pregnancy	0 (0%)	1 (2.5%)	0.365 NS
Ectopic Pregnancy	0 (0%)	0 (0%)	NE

IUI intrauterine insemination
Data presented as frequency (percentage)
1 Analysis using chi-squared test
RR (95% CI) risk ratio and its 95% confidence interval
S significant – NS non-significant – HS highly significant
NE not estimable due to nullity in all groups