IRAQI JOURNAL OF EMBRYOS AND INFERTILITY RESEARCHES (IJEIR)
Kinanah et al., Vol. 10, Issue 2, Pp. 25-50 (2020) Original Research
DOI: http://doi.org/10.28969/IJEIR.v10.i2.r3.20 E-ISSN: 2616-6984 P-ISSN: 2218-0265

Impact of Endometrial flushing of human Chorionic Gonadotrophin on pregnancy rates in intrauterine insemination cycles.

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ABSTRACT

Intrauterine insemination (IUI) comprises the insertion of a high number of washed and processed spermatozoa directly into the cervix to the uterine cavity around the time of ovulation to increase the chances of pregnancy. It is usually used for couples with mild male factor infertility, unexplained infertility, cervical factors, mild endometriosis, and psychological, sexual dysfunction. The endometrium, which lines the uterus, is prepared for the implantation of the embryo; this process starts in the proliferative phase and extends throughout the luteal phase. Implantation, a crucial phase of pregnancy, is a complicated process, and more than half of failures to achieve pregnancy are caused by implantation failure. To assess the influence of endometrial flushing of 1000IU hCG done prior to IUI procedure on pregnancy rate in infertile females undergoing IUI treatment. The present study was applied to 80 infertile subjected to ovarian stimulation protocol using letrozole and hMG in doses adjusted according to their response; triggering was done using subcutaneous HCG. The results of the present study showed no significant difference regarding initial characteristics, indication for IUI, basal menstrual cycle characteristics, ovarian stimulation characteristics, and semen parameters before and after processing, while there was a significant difference between both groups regarding biochemical and clinical pregnancy rates. In IUI cycles, endometrial human chorionic gonadotropin (hCG) flushing done 5-10 minutes prior to IUI can improve biochemical and clinical pregnancy rates.

Received: 07-July-2020
Accepted: 30-July -2020
Published: 08-Aug-2020

How to cite:

Kinanah I. S. Al-Tamemi , Mufeda A. Al-Ammar , Muayad S. Abood; Impact of Endometrial flushing of human Chorionic Gonadotrophin on pregnancy rates in intrauterine insemination cycles; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2020); 10 (2): 25-50.

Doi: http://doi.org/10.28969/IJEIR.v10.i2.r3.20

KEYWORD

IUI, hCG endometrial flushing, Pregnancy rate, Infertility.

1. Introduction

Intrauterine insemination (IUI) is a cheap, not invasive first-line treatment option for certain infertile couples with patent fallopian tubes; it is indicated for cervical factor infertility, male infertility except for severe male factors, unexplained infertility, anovulation, infertility due to immune causes and also for disorders in the ejaculation process. IUI yields a clinical pregnancy rate fluctuating between 10% to 20% per cycle. However, IUI may have a restricted role in severe males complaining of infertility caused by several issues, including endometriosis, infertility due to diseased fallopian tubes, and in females ≥ 35 years old (Allahbadia, 2017 [1]).

The Intrauterine insemination (IUI) cycle's success is dependent on various factors that include endometrial receptivity, the responsiveness of the ovary if stimulated, maternal age, a higher number of mature preovulatory follicles, and seminal fluid parameters (Veltman-Verhulst *et al.*, 2012 [2]). Implantation of the embryos, which is an essential event for a successful pregnancy, can only occur in a receptive uterus (Gnainsky *et al.*, 2010 [3]). Successful implantation necessitates a competent embryo, a receptive endometrium, and a coordinated dialogue between maternal and embryonic sides (Rubel *et al.*, 2010 [4]). Implantation is a complex process that is synchronized by several autocrine and paracrine factors, and hCG is one of the most important. Competent embryos secrete hCG earlier to implantation (Wirleitner *et al.*, 2015 [5]), and the start of hCG transcription starts from the two-cell stage embryo; it is also secreted from the endometrial epithelial cells, mostly through a luteal phase of the ovarian cycle (Bourdiec *et al.*, 2013 [6])

Human Chorionic Gonadotropin (hCG), also known as "the hormone of pregnancy," has fundamental characteristics in human reproduction.

Some researchers had revealed its essential roles in establishing and preserving the pregnancy from fertilization, placenta formation, initial embryo growth, and until the end of pregnancy (Theofanakis et al., 2017 [7]). Human Chorionic Gonadotropin also supports trophoblast apposition (loose arrangement of the trophoblasts to the decidua) and adhesion (closer connection of the trophoblasts to the decidua) to the endometrium by regulating proteins required for implantation (Racicot KE et al., 2014 [8]). In normal sexual intercourse and after ejaculation, sperms are bound to the ciliated epithelial cells in the isthmus part of fallopian tubes, and they are exposed to secretions of epithelial cells with the purpose of hyperactivation and capacitation that it is so essential for fertilization events. Human Chorionic Gonadotropin (hCG) is secreted by both endometrial cells and fallopian tubal cells that may be crucial for sperm motility and subsequent fertilization (Moosavi SM et al., 2018 [9]). In brief, various mechanisms have been postulated in which hCG can regulate the process of implantation. An in vitro study showed that hCG is a strong attractor of cells of inflammation, like neutrophils, lymphocytes, and monocytes. hCG directly regulates the response of endothelial cells to interleukin one and then augments the cytokine-mediated consequence on the cell's proliferation migration and leads to the release of various factors (Fahmy A A et al., 2016 [10]).

Intrauterine flushing of hCG around the time of ET has the potential to improve the outcome of assisted reproduction modalities, and randomized and nonrandomized trials have described variable results (Rebolloso M M et al., 2013 [11]).

2. Materials and Methods

This randomized prospective comparative study was performed in the High Institute for Infertility

Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University/ Baghdad/ Iraq during the time period between November 2020 and May 2021.

This is study included 80 infertile couples who attended the ART unit of the institute and had been scheduled for IUI. All infertile couples were subjected to a full history taking, complete general and gynecological examination, and full infertility investigations, including the husband's seminal fluid analysis, early follicular phase hormonal profile, a transvaginal ultrasound, and hystrosalpingography for evaluation of uterine cavity and tubal patency and/or laparoscopy for evaluation of tubal patency and exclusion of any 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 this study, all of the patients were counselled, and written informed consents were obtained from all of the participants, and 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 the females should have a normal thyroid function test and normal blood sugar levels.

The 80 infertile females were selected randomly and divided into two groups:

Group 1: The control group consisted of (40) infertile females who received standard ovarian stimulation (letrozole and/or gonadotropins) and underwent IUI without endometrial human Chorionic Gonadotropin flushing.

Group 2: The study group and consisted of (40) infertile females; they received standard ovarian stimulation (letrozole and/or gonadotropins) and underwent IUI with endometrial human Chorionic Gonadotropin flushing 1000 IU done 5-10 minutes prior to the IUI procedure. Letrozole tablet (2.5 mg) was given twice per day from the third day or 5th day of the menstrual cycle and for five days. Recombinant FSH 75 IU / vial or hMG injections 75 IU of both urinary FSH and LH it was given from day 5 of the cycle and the dose was adjusted according to the patient's response. Maturity of the follicles was monitored by serial transvaginal ultrasound scans to evaluate follicles number, size and endometrial thickness. The size of the dominant follicle should be between 17-23 mm to give the ovulation trigger. On day 12 or 13 and when at least one follicle reaches 17 mm, 6500IU /vial(250mg) of recombinant human Chorionic Gonadotropin (hCG) was given subcutaneously.

The preparation of the intrauterine injection of hCG consisted of 1000 IU of hCG (0.4ml) and 0.1 mL of normal saline, so the total volume was 0.5ml. In the present study, the dilution was prepared to obtain a dose of 1000 IU of hCG with the lowest level of sterile saline. Uterine flushing by hCG was performed for group 2. The endometrial human Chorionic Gonadotropin flushing was done 5-10 minutes before IUI in group 2.

At the of IUI and shortly before the procedure, the women in group 2 were asked to lie in the lithotomy posture, and the uterine cervix was visualized with a vaginal speculum; firstly, removal of excess vaginal and cervical secretions with a sterile gauze was done, then intrauterine insemination cannula was introduced into the uterine cavity through 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 5-10 minutes post endometrial hCG flush. Post-IUI luteal phase support was given in the form of progesterone suppositories to all patients for fourteen days.

Pregnancy was detected by serum β-hCG test performed 14 days after IUI for all patients. If the serum β-hCG test was positive, vaginal ultrasound was organized to be done two weeks later to confirm the presence of an intrauterine gestational sac.

3. 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 1 and a mean age of 31.18 ± 6.52 years for group 2. 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 1, in the range of 21.6 – 40.0 kg/m^2 with a mean of 29.93 ± 4.33 for group 2.

The parity was in the range of 0 – 5 for group 1 and in the range of 0-4 for group 2, with a median of 0 for both groups.

The number of previous miscarriages was in the range of 0-3 for group 1 and 0-6 for group 2, 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 1, while 50 % of women had primary infertility while 50 % of them had secondary infertility in group 2.

The duration of infertility was in the range of 1-14 years and a median of 5 years in group 1, but it was in the range of 1-10 years and a median of 6 for group 2. 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 1, 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 2, the indications of IUI were 35% for male factors, 22.5% for anovulation (PCOS), 5% for unilateral tubal block, 2.5 % for mild endometriosis, 30 % for unexplained infertility, and 5% for combined male and female Factors **(Table- 2).**

3- Difference between groups regarding basal cycle characteristics

Cycle regularity and hormonal analysis comparison for all females enrolled in the present study was done on the second or third day of the menstrual cycle, as shown in **(Table–3)**. The menstrual cycle was regular in 65% of women in Group 1 and in 77.5% of women of Group 2. The basal FSH in mIU/L was in the range of 2.4 – 10.1 mIU/L and a median of 7.6 in group 1, in a range of 3.0 – 10.2 mIU/L and a median of 7.5 in group 2.

The basal LH in mIU/L was in the range of 1.8 – 11.9 mIU/L and a median of 6.7 in group 1, in a range of 1.3 – 15.2 mIU/L and a median of 5.3 in

group 2. The basal estradiol level in (pg/ml) was in the range of 13.4 – 51.0 pg/ml and a median of 43 in group 1, in a range of 12.0 – 52.3 pg/ml and a median of 33.1 in group 2. 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, r-FSH, letrozole + HMG, or letrozole + r-FSH. In both groups, letrozole was used in fifty percent or more of the cases.

In group 1, letrozole was used in 60% of women, HMG in 12.5%, r-FSH in 2.5%, letrozole + HMG in 20% and letrozole + r-FSH in 5% of women. In group 2, letrozole was used in 50 % of women, HMG in 12.5%, r-FSH in 0%, letrozole + HMG in 35% and letrozole + r-FSH in 2.5% of women. The number of mature follicles was in the range of 1-4 for both groups 1 2, with a median of 2 for both groups. The maximum dimension of the dominant follicle in (mm) was 18-24 mm for group 1 and 18-23 for group 2, with a median of 20 mm for both groups.

The mid-cycle estradiol levels in (pg/ml) were highest in group 2. The mid-cycle estradiol levels were in the range of 108 – 603 pg/ml and in a median of 284 pg/ml in women enrolled in group 1, in the range of 79 – 849 pg/ml, and in a median of 311 ng/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 – 11.0 mm and a median of 8.8 in groups 1 and 2, respectively.

There were no significant differences between women of the two groups regarding ovarian stimulation drugs, number of mature follicles, the maximum dimension of the dominant

follicle, midcycle serum estradiol level, or peak endometrial thickness **(Table 4).**

5- Difference between Groups regarding Semen Characteristics before and after Processing.

Semen Volume (ml) is in the range of 1.0 – 3.5 ml for group 1, 1.0 – 5.0 ml for group 2, and a median of 2.0 for both groups. For group 1, before processing, sperms concentration (x 10^6/mm) was in the range of (6.6 – 62.0) 10^6/mm, and median of 21.0 and

after processing changed to a range of (7.0 – 45.0)10^6/mm and a median of 15.0, Progressive Motility (%) was in range of (5.0 – 75.0) % and a median of 35.5 before processing and changed to a range (38.0 – 100.0) % and a median of 79.5 after it. Normal Morphology (%) was in the range of (4.0 – 85.0) % and a median of 45.0 before processing and changed to a range of (22.0 – 95.0) % and a median of 85.5 after it. For group 2, before processing, sperms concentration (x 10^6/mm) was in the range of 6.0 – 97.0 10^6/mm, and a median of 29.0 and after processing changed to a range of (7.0 – 45.0) 10^6/mm and a median of 15.0, Progressive Motility (%) was in range of (5.0 – 70) % and a median of 35.0 before processing and changed to a range (40.0 – 100.0) % and a median of 77.5 after it. Normal Morphology (%) was in the range of (5.0 – 95.0) % and a median of 40.0 before processing and changed to a range of (10.0 – 100.0) % and a median of 80.0

after it. There were no significant differences between women of the three groups regarding semen parameters before and after processing (table 5).

6- Difference between Groups regarding Pregnancy Outcomes:

Both Biochemical and clinical Pregnancy rates were the highest in group 2; the biochemical pregnancy rate was 7.5% and 30%, while the clinical pregnancy rate was 5% and 27.5% in groups 1 and 2, respectively;

multiple pregnancy only had been seen

in group.

Table (1): Difference between Groups regarding Initial Characteristics.

	Group 1 Control Group (n=40)	**Group 2 hCG Flushing on the day of IUI (n=40)**	***P***
Age (years) Range Mean ± SD	20 – 40 30.65 ± 5.17	19 – 40 31.18 ± 6.52	0.917 NS
BMI (kg/m^2) Range Mean ± SD	18.4 – 39.1 28.53 ± 4.99	21.6 – 40.0 29.93 ± 4.33	0.342 NS
Parity Range Median (IQR)	0 – 5 0 (0 – 2)	0 – 4 0 (0 – 2)	0.766 NS
No. of Previous Miscarriages Range Median (IQR)	0 – 3 0 (0 – 1)	0 – 6 0 (0 – 1)	0.431 NS
Type of Infertility Primary Secondary	17 (42.5%) 23(57.5%)	20 (50.0%) 20 (50.0%)	0.531 NS
Duration of Infertility (years) Range Median (IQR)	1 – 14 5 (3 – 7)	1 – 10 5 (3 – 7)	0.183 NS
No. of Previous IUI Cycles Range Median (IQR)	0 – 3 0 (0 – 2)	0 – 4 0 (0 – 1)	0.113 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)

NS non-significant

Table (2): Difference between Groups regarding Indication for IUI.

	Group 1 Control Group (n=40)	**Group 2 hCG Flushing on the day of IUI (n=40)**	***P***
Indication for IUI **Male Factor** **Anovulation** **Unilateral Tubal Block** **Endometriosis** **Unexplained Infertility** **Combined Male and Female Factors**	 11 (27.5%) 13 (32.5%) 3 (7.5%) 1 (2.5%) 7 (17.5%) 5 (12.5%)	 14 (35%) 9 (22.5%) 2 (5%) 1 (2.5%) 12 (30%) 2 (5%)	 0.586 NS
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.

	Group 1 Control Group (n=40)	**Group 2 hCG Flushing on the day of IUI (n=40)**	***P***
Menstrual Regularity **Regular** **Irregular**	 26 (65%) 14 (35%)	 31 (77.5%) 9 (22.5%)	 0.104 NS
Basal FSH (mIU/L) **Range** **Median (IQR)**	 2.4 – 10.1 7.6 (5.3 – 9.3)	 3.0 – 10.2 7.5 (5.9 – 8.8)	 0.936 NS
Basal LH (mIU/L) **Range** **Median (IQR)**	 1.8 – 11.9 6.7 (4.3 – 7.9)	 1.3 – 15.2 5.3 (3.7 – 8.3)	 0.365 NS
Basal Estradiol (pg/ml) **Range** **Median (IQR)**	 13.4 – 51.0 43 (41.6 – 43.8)	 12.0 – 52.3 33.1 (29.0 – 43.1)	 0.067 [2] NS
IUI intrauterine insemination – IQR interquartile range FSH follicle stimulating hormone – LH luteinizing hormone Data presented as frequency (percentage) or range, median (IQR)			

Table 4: Difference between Groups regarding Ovarian Stimulation Characteristics.

	Group 1 Control Group (n=40)	**Group 2 hCG Flushing on the day of IUI (n=40)**	***P***
Ovarian Stimulation Drugs **Letrozole** **HMG** **r-FSH** **Letrozole + HMG** **Letrozole + r-FSH**	24 (60%) 5 (12.5%) 1 (2.5%) 8 (20%) 2 (5%)	20 (50%) 5 (12.5%) 0 (0%) 14 (35%) 1 (2.5%)	0.064 [1] NS
No. of Mature Follicles **Range** **Median (IQR)**	1 – 4 2 (1 – 2)	1 – 4 2 (1 – 2)	0.129 [2] NS
Max Dimension of Dominant Follicle (mm) **Range** **Median (IQR)**	18 – 24 20 (19 – 21)	18 – 23 20 (19 – 21)	0.471 [2] NS
Midcycle Estradiol (pg/ml) **Range** **Median (IQR)**	108 – 603 248 (194 – 342)	79 – 849 311 (240 – 498)	0.104 [2] NS
Peak Endometrial Thickness (mm) **Range** **Median (IQR)**	8.1 – 11.5 9 (8.4 – 9.2)	8.2 – 11.0 8.8 (8.2 – 9.3)	0.951 [2] NS

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

Table (5): Difference between Groups regarding Semen Characteristics before and after processing

		Group 1 Control Group (n=40)	Group 2 hCG Flushing on the day of hCG Trigger (n=40)	*P* [1]
Semen Volume (ml) **Range** **Median (IQR)**		1.0 – 3.5 2.0 (1.5 – 2.5)	1.0 – 5.0 2.0 (2.0 – 2.9)	0.336 NS
Before Process -ing	**Sperm Concentration (x 10^6/mm)** **Range** **Median (IQR)**	6.6 – 62.0 21.0 (15.2 – 30.0)	6.0 – 97.0 29.0 (15.3 – 47.3)	0.128 NS
	Progressive Motility (%) **Range** **Median (IQR)**	5.0 – 75.0 35.5 (25.0 – 48.8)	5.0 – 70.0 35.0 (20.0 – 45.0)	0.545 NS
	Normal Morphology (%) **Range** **Median (IQR)**	4.0 – 85.0 45.0 (31.0 – 55.0)	5.0 – 95.0 40.0 (26.3 – 63.8)	0.245 NS
After Process -ing	**Sperm Concentration (x 10^6/mm)** **Range** **Median (IQR)**	7.0 – 45.0 15.0 (10.0 – 19.5)	5.0 – 68.0 20.0 (13.0 – 25.0)	0.069 NS
	Progressive Motility (%) **Range** **Median (IQR)**	38.0 – 100.0 79.5 (70.0 – 90.0)	40.0 – 100.0 77.5 (70.0 – 88.8)	0.118 NS
	Normal Morphology (%) **Range** **Median (IQR)**	22.0 – 95.0 85.0 (75.0 – 88.0)	10.0 – 100.0 80.0 (60.0 – 90.0)	0.174 NS

IUI intrauterine insemination – IQR interquartile range
Data presented as range, median (IQR)
1 Analysis using the Kruskal-Wallis test
NS non-significant

Table (6): Difference between Groups regarding Pregnancy Outcomes.

	Group 1 Control Group (n=40)	Group 2 hCG Flushing on the day of IUI (n=40)	P [1]
Biochemical Pregnancy	3 (7.5%)	12 (30.0%)	0.003 S
Clinical Pregnancy	2 (5.0%)	11 (27.5%)	0.001 S
Multiple Pregnancy	0 (0%)	0 (0%)	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 is not estimable due to nullity in all groups

4. Discussion:

Three constituents are believed to be crucial for successful implantation: embryo quality, endometrial receptivity, and embryo–endometrium communication (Hou W et al., 2012 [12]). Implantation is coordinated by many factors, and the most important factor in this regard is human Chorionic Gonadotropin (hCG). It is the final consequence of complicated molecular interactions between a primed uterus and a mature blastocyst (Firouzabadi D R et al., 2013 [13]). Human Chorionic Gonadotropin (hCG) is involved in many processes ensuring the smooth progress of pregnancy: the recognition of the pregnancy by the maternal organism, the maintenance of the corpus luteum, the stimulation of progesterone production, the strengthening of the implantation of

the embryo, angiogenesis, and vasculogenesis necessary for placental development, control of trophoblast

differentiation, and finally, immune regulation in the maternal/ fetal interface (Gridelet V et al., 2014 [14]).

In this randomized prospective comparative study, two groups were studied; intrauterine insemination (IUI) was done for infertile females in group 1 without any intervention, while study group 2 was subjected to 1000 IU endometrial hCG flush before IUI, it was tried to examine the effects of endometrial hCG flushing on the outcome of the IUI cycles. It was found that intrauterine hCG flushing before IUI significantly improves the biochemical and clinical pregnancy rates. The results indicate that the flushing of 1000 IU shortly before the IUI procedure would improve the outcomes .A comparison was done between control and study groups concerning clinic-demographic parameters, initial characteristics, indication for IUI, basal cycle characteristics, ovarian stimulation characteristics, and semen characteristics before and after processing. The statistical analysis revealed no significant difference, and this is gain is mandatory from a statistical perspective as it is essential in such a randomized controlled study to ensure the absence of any factors that possibly affect positively or negatively the pregnancy outcomes .

The biochemical and clinical pregnancy rates were significantly superior in women of group 2 when compared to women of group 1. There were no significant differences between women of both groups concerning multiple pregnancy. None of the included women in either group had ectopic pregnancy .

The novel practice of intrauterine hCG flushing was first carried out by Mansour and colleagues (Mansour R et al., 2011 [15]). They established that 1 mL of culture media containing 500 IU of hCG mirrors the ideal concentration to be introduced into the uterine cavity about 7 minutes prior to embryo

transfer (ET), promising improved pregnancy rates (Makrigiannakis A et al., 2017 [16]).

However, there are several dissimilarities in studies comprising intrauterine hCG administration before either blastocyst transfer or earlier embryo transfer, the type of hCG used was either urinary or recombinant, and also hCG was used in many concentrations and to different time-points earlier to embryo transfer (Makrigiannakis A et al., 2017 [16]).

Also, several researchers as Zarei et al. (Zarei A et al., 2014 [17]); Santibañez et al. (Santibañez et al., 2014 [18]); Ye et al. (Ye H et al., 2015 [19]); Craciunas et al., (Craciunas L et al., 2016 [20]); Hosseini et al., (Hosseini RS et al., 2016 [21]), Gao et al., (Gao M et al., 2019 [22]); Tan et al., (Tan H et al., 2019 [23]) and very recently Asbagh et al., (Asbagh P A et al.,

2021 [24]) found that infusion of hCG at different times and by different doses before embryo transfer improved the IVF/ICSI outcomes. Navali et al. (Navali N et al., 2016 [25]) confirmed that intrauterine flushing of 500 IU of hCG during general anesthesia after oocyte pickup could efficiently improve both the implantation and pregnancy rates (Navali N et al., 2016 [25]).

In contrast, studies accomplished by Wirleitner et al., (Wirleitner et al., 2015 [5]); Firouzabadi et al., (Firouzabadi D R et al., 2013 [13]); Osman et al., (Osman A et al., 2016 [26]) and Hou et al., (Hou W et al., 2012 [12]) reported dissimilar results and suggested that the use of intrauterine hCG flushing prior to embryo transfer (ET) does not improve ICSI outcomes. Notably, Volovsky et al. (Volovsky M et al. 2018 [27]) even revealed undesirable effects on clinical pregnancy rates after intrauterine hCG flushing and

this was mostly true for females without clear, repeated implantation failure (RIF) (Volovsky M et al., 2018 [27]).

Regarding setting the control group without any intervention (as normal saline or culture media flush), is going agree with Fahmy (Fahmy A A et al., 2016 [10]) and his colleagues in their only study about the effect of hCG flush on IUI outcome, also agrees with many researchers in IVF/ICSI cycles as Mansour, (Mansour R et al., 2011 [15]); Leao et al., (Leao RBF et al., 2013 [28]), Cambiaghi et al., (Cambiaghi AS et al., 2013 [29]); Janati et al., (Janati S et al., 2013 [30]); Santibañez et al., (Santibañez et al., 2014 [18]); Kokkali et al., (Kokkali G et al., 2014 [31]); Firouzabadi et al., (Firouzabadi D R et al., 2013 [13]). And very recently with Asbagh and his colleagues (Asbagh P A et al., 2021 [24]). This study disagrees with many researchers, such as Zarei et al. (Zarei A et al., 2014 [17]); Wirleitner et al. (Wirleitner et al., 2015 [5]) and Navali et al. (Navali N et al., 2016 [25]). Who considered females that had intrauterine normal saline or culture media flush as control groups ?

It was hypothesized that the achievement of physiological properties of hCG required convincing physiological doses in a well-timed fashion, which should take into account the phases of either embryonic development or endometrial receptivity (Butler SA et al., 2013 [32]). The hCG concentrations of 100 IU and 200 IU, however, did not improve the pregnancy rates (Mansour R et al., 2011 [15]).

From these previous studies, the selection of a dose of 1000 IU hCG agrees with (Janati S et al., 2013 [30]); and Firouzabadi (Firouzabadi D R et al., 2013 [13]). Evidence revealed a significant rise in the implantation rate among infertile females who received more than 500 IU intrauterine hCG (Tan H et al., 2019 [23]).

In the current study, the chosen volume for intrauterine flushing, which was 0.5 ml, is the same as in the previous study, which supposed that by directing this volume, not only more of the endometrial surface would get the drug

but also the drug would have time to prompt the pathway towards better receptivity (Zarei A et al., 2014 [17]).

The hCG endometrial flushing programs were diverse among the studies, and the investigators' absence of clarifications for the elected times was so confusing. The precise timing of hCG infusions included less than 3 minutes, 7 minutes, 5–7 minutes, 12 minutes, and immediately following oocyte pick up (Hou W et al., 2012 [12]).

It may be probable that the ideal practice included hCG flushing at a minimum dosage of 500 IU 5 to 12 minutes prior to the cleavage stage embryo transfer (Simopoulou M et al., 2019 [33]).

Whether hCG's influences on the endometrium and embryos was transitory remains unclear. Therefore, the time of hCG flushing might be an essential variable affecting IVF/ICSI outcomes (Hou W et al., 2012 [12]).

There is no doubt that hCG drugs possess a diversity of immune-modulating properties (Schumacher A et al., 2014 [34]). However, hCG's mechanism in supporting the immune system in its function in upregulating embryo implantation and placentation is far from being understood. The contribution of immune cells in pregnancy-related processes is not limited to pregnancy itself but initiates during the menstrual cycle before ovulation occurrence (Schumacher A & Zenclussen A C et al., 2019 [35]).

It was previously acknowledged for more than two decades that LH/hCG receptors are expressed in the human uterus (Reshef E et al., 1990 [36]). These receptors are present in various cell types, particularly in epithelial cells (Rao C V, 2001). Based on this knowledge, it can be assumed that hCG could improve endometrial receptivity via the improvement of endometrial quality and stromal fibroblast function (Theofanakis et al., 2017 [7]).

Because of these reports and the previous findings about there being a benefit to uterine angiogenesis and the

inflammatory process, both mechanisms reflect the possible effects of hCG directly on the endometrium in IUI cycles instead of having an autocrine role on human embryos, as seen in IVF/ICSI cycles (Fahmy A A et al., 2016 [10]). This hormone induces also the upregulation of aromatase expression and estradiol production in human granulosa lutein cells. hCG strongly stimulates the expression of its own receptor in human luteal cells (Convissar S et al., 2019 [38]).

The explanation of the beneficial effect of intrauterine hCG is going ahead with all previously mentioned mechanisms added to their hCG influence on the sperms that are present in the semen suspension, which are expelled into the uterine cavity .

Subsequent to ovulation, the sperms are detached from the fallopian tubes epithelial cells and start to travel toward the released oocyte in ampulla for fertilization, so it seems that secretions of fallopian tubal cells are necessary for sperms hyperactivation and motility (Coy P et al., 2012 [39]). hCG is secreted by fallopian epithelial cells and endometrial cells and may play a positive role on the hyperactivated motility of sperms and subsequent fertilization (Moosavi SM et al., 2018 [9]).

The hCG/LH receptors are located on corpus luteal cells to promote progesterone secretion, on the decidua for initial contact with the blastocyst, on myometrium for growth coordination between fetus and uterine size, on umbilical cord tissue for growth, on uterine vasculature for angiogenesis, on cytotrophoblast cells for differentiation, and also on various fetal organs for both growth and differentiation (McPherson R A & Pincus M R, 2006 [40]).

The hCG/LH receptors are observed in human sperms and in the fallopian tubes, seen by the manifestation of mRNA and demonstration of receptors' actions. Really, the function of the hCG/LH receptors in human sperms is not clear; perhaps it has some

association to fertility statutes. The hCG/LH receptors in the fallopian tubes may be controlled by the LH hormone, which in turn relaxes the fallopian tubes to prepare for fertilization (Krzywy-Gawrońska E, 2010 [41]).

A fact may propose that the hCG actions might be wider than once thought to be; this is achieved by profuse distribution of hCG receptors in reproductive tissues, chiefly in the gonads, ovary, and testis, and also be found

in extra-gonadal reproductive organs, such as the uterus, the fallopian tubes (Theofanakis et al., 2017 [7]). And in the human sperm (Eblen A et al., 2001 [42]).

It seems that this "hormone of pregnancy" has more treatment modalities and applications than once thought, and one can only hope that future studies will reveal even more talented usages of this old-new hormone.

Acknowledgment

We would like to acknowledge the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/ Al Nahrain University, Baghdad, Iraq.

Funding

This work received no funding .

Author Contribution

Kinanah I. S. Al-Tamemi, performed the study, Mufeda A. Al-Ammar, Muayad S. Abood, supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The Ethical Approval Committee approved the study.

Financial Disclosure

There is no financial disclosure.

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