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The Impact of Preovulatory Human Chorionic Gonadotrophin Uterine Flushing on Implantation Rate in Women with Unexplained Infertility Scheduled For Intra-Uterine Insemination: A Randomised Prospective Study

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Abstract

To evaluate the impact of preovulatory human chorionic gonadotrophin (HCG) uterine flushing on implantation rate in women with unexplained infertility receiving intrauterine insemination (IUI).This was a prospective study performed at Ain Shams University Maternity Hospital, over a 3-year period, between June 2012 and June 2015, and included 300 women who were presented at the infertility clinic and scheduled for having IUI. Participant ages ranged from 18 to 37 years, the patients were divided randomly into two equal groups; group I (test group, N=150) included women who had preovulatory HCG endometrial flush followed by IUI and the other group (control group, N=150) who had IUI only without HCG endometrial flushing. The outcome of interest is the difference in the rate of biochemical and clinical pregnancies, resulting from one cycle of treatment, between the two intervention groups. The outcomes of 300 cycles were analysed, the biochemical and clinical pregnancy rates in group I (test group) were (27.3%, 22% respectively while in group II (control group) were21.3%, 16% respectively with significant difference between the two groups (P<0.05), (44.7%) of patients experienced mild discomfort, moderate discomfort in (28.7%), severe discomfort in (11.3%), also patient acceptability was high (68%). Difficult flushing was present in 14.7% of cases. The side effects were low in general but higher than in group II with significal difference between the two groups (nausea was present in 21.3% of cases of group I Vs 6.7 in group II, vomiting in 10% in group I

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Vs 2% in group II, abdominal cramps 37.3% in group I Vs 6% in group II , vaginal bleeding 12.7 in group I Vs 2.7 in group II). There were no long term complications of the intervention.

Keywords

Human chorionic gonadotrophin; uterine flush; unexplained infertility; intrauterine insemination

I. Introduction

Infertility is the inability to conceive after one year or more of regular coitus with no contraception. Epidemiological researches have reported that about 80% of couples had conceived during that period. It is postulated that nearly 15% of couples are infertile in developed nations [1]. There has not been reported a substantial rise in demand for the treatment of infertility in the last decade [2]. Implantation, of the embryo, depends on the embryo quality and the endometrial receptivity. It is estimated that failure of implantation accounts for approximately 50% to 75% of lost pregnancies [3].

Intrauterine insemination (IUI) is a common therapy and during 2001-2004 in Europe, the conception rate in IUI cycles had ranged between 11.4% and 12.6% [4] and the rate of multiple births between 11.2% and 13.1%. As shown by the ESHRE Capri Workshop Group on IUI [4], inspite of the utilization of induction of ovulation programs and the manipulation of semen samples, the conception rates in IUI cycles are not significantly higher than the results produced after ordinary or timed coitus. Actually, IUI had not been considered as an assisted reproductive technique (ART) inspite of its common use [5]. The ESHRE Capri Workshop Group reported the role of individual topics in the efficacy of IUI therapy. One of the topics was the insemination time which was done 32-36 hours after HCG injection [4]. However, it looks that among healthy women, the best time to become pregnant is if coitus occurred up to six days before ovulation [6].

HCG is a placental heterodimeric glycoprotein hormone that is needed to maintain conception. HCG is initially synthesized by the blastocyst 6–8 days postconception [7,8]. Before implantation, there is an early production of HCG by embryos of primates. Various mechanisms had 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 [9]. HCG directly regulates the response of endothelial cells to interleukin 1 and enhances the cytokine-mediated effect on the proliferation of cells, migration and release of many factors [10]. Our aim was to reproduce and confirm the impact of intrauterine injection of HCG before embryo transfer on the conception rates in the IUI cycles at our IVF center.

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II. Methods

This was a prospective study performed at Ain Shams University Maternity Hospital, over a 3-year period, between June 2012 and June 2015, and included 300 women who were presented at the infertility clinic and scheduled for having IUI. The patients were divided randomly into two equal groups; group I (test group, N =150) included women who had preovulatory HCG endometrial flush followed by IUI and the other group (control group, N=150) who had IUI only without HCG endometrial flushing.

Inclusion criteria:

1. Primary or secondary infertility ≥ one year
2. Participant age: 18 - 37
3. Diagnosis of unexplained infertility ≤ 36 months

Anti-Müllerian hormone ≥ 0.4 ng/mL and/or follicle stimulating hormone ≤13 IU/L in early follicular phase

Regular cycle of 25–35 days, positive ovulation tests, and/or midluteal progesterone ≥25 mmol/L in an unstimulated cycle

Normal semen analysis according to WHO 2010 criteria

No uterine cavity abnormalities

Normal Fallopian tubes

4. Negative genitourinary test for chlamydia and gonorrhea ≤ one year

Exclusion criteria

1. Body mass index (BMI) ≥35 kg/m2
2. Ongoing conception

All included women were subjected to revising history and examination sheets with particular emphasis on personal history: age, residence, education level and socioeconomic status, Complaint regarding infertility, obstetric history including parity and gravidity and ultrasound for any uterine or tubal abnormality, the number of ovarian follicles and the diameter of the dominant follicle. The endometrium was measured at the greatest anterioposterior dimension under a longitudinal section.

A simple computer-generated randomisation was done by an independent statistician in a ratio of 1:1 and transferred into sealed opaque envelopes.

All women received the same ovarian hyperstimulation protocol: combined clomiphene citrate (CC) and human menopausal gonadotropin (hMG) (Pergonal, Serono, Rome, Italy). An oral dose of CC (200 mg/ day) was given on cycle day 2 through cycle day 6 and three doses of HCG (150 IU/day) were administered on cycle day 7, 9, and 11. Follicular survey was done on cycle day 8, 11, and 13.

Uterine flush by HCG was performed preovulatory (when two follicles > 16 mm) by removal of excess vaginal and cervical secretions with a cotton swab, a 5 Fr intrauterine insemination cannula was introduced into the uterine cavity through the cervical canal, preferably without the use of a volsellum. Then, 10 mL of HCG (5000 IU) in sterile saline were injected intrauterine through the cannula, no premedication was given. In case of pelvic pain, women were asked to take paracetamol if needed and avoid anti-inflammatory drugs. HCG (5000 IU) was also given intramuscularly.

The semen was prepared with Enhance (Percoll) method using three different density

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(95%, 70%, 50% Percoll) gradient centrifugation. All patients received IUI 34–36 hr after HCG injection.

Progesterone was given since day 3 post-IUI. All Clinical pregnancy was detected as a positive urine pregnancy test 2 weeks post-IUI and confirmed by transvaginal ultrasonography of intrauterine gestational sac. If a pregnancy occurred, women were advised to continue the aspirin 81 mg through 6 weeks after IUI.

The outcome of interest is the difference in the rate of biochemical and clinical pregnancies, resulting from one cycle of treatment, between the two intervention groups. Adverse effects were reported. After completing the intervention, the clinician document adds the following symptoms: pain (none, mild, moderate, severe), nausea, vomiting, weakness, dizziness or loss of consciousness. At 1 month, participants will be asked if they have experienced fever, pelvic infection or any other late adverse effects in a phone interview. They were also inquired about uterine flushing as an acceptable treatment option and if they would be willing to repeat this treatment for a new cycle.

The hospital ethics committee approved the study. All patients gave their informative consent before entering into the study.

Statistical analysis: Retrieved data were recorded on an investigative report form. The data were analyzed with SPSS® for Windows®, version 15.0 (SPSS, Inc, USA). Description of quantitative (numerical) variables was performed in form of mean, standard deviation (SD) and range. Description of qualitative (categorical) data was performed in the form of numbers and percent. Analysis of numerical variables was performed by using student's unpaired t-test (for two groups) or ANOVA (for more than two groups). Analysis of categorical data was performed by using Fischer's exact test and Chi-squared test. Logistic regression analysis was performed to calculate association between variables and their odds ratios. Association between variables was estimated using Pearson's correlation coefficient (for parametric variables) and Spearman's correlation coefficient (for non-parametric variables). Significance level was set at 0.05.

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III. Results

The current study was conducted on 300 women presented at infertility clinic of Ain Shams Maternity Hospital, during the period between June 2012 and June 2015. The study included 2 groups of women: group I [n=150]; women who had preovulatory HCG uterine flush then IUI, and group II [n=150]; women who had underwent IUI without preovulatory HCG endometrial flush. There was no statistically significant difference between the two groups concerning the clinic-demographic parameters including mean age, menarche age, BMI, mean gravidity, duration and type of infertility, educational level, occupation, number of developing follicles at time of insemination, mean diameter of dominant ovarian follicle and mean endometrial thickness (table 1).

Table (2) shows a comparison between the two studied groups as regards the biochemical and clinical pregnancy rates. In group I (test group) the biochemical pregnancy rate was 27.3% and clinical pregnancy rate was 22% while in group II (control group) the biochemical pregnancy rate was 21.3% and clinical pregnancy rate was 16% with statistically significant difference between both groups (P <0.05)

As regards the patient compliance and experience of discomfort in cases of group I, the patients experienced mild discomfort in (44.7%), moderate discomfort in (28.7%), and severe discomfort in (11.3%), also patient acceptability was (68%). Difficult flushing was present in 14.7% of cases (table 3).

The development of side effects were evaluated in table (4): they were low in general but higher than group II with significal difference between the two groups (nausea was present in 21.3% of cases of group I Vs 6.7% in group II, vomiting in 10% in group I Vs 2% in group II, abdominal cramps 37.3% in group I Vs 6% in group II , vaginal bleeding 12.7 in group I Vs 2.7 in group II,). There were no long term complications of the intervention.

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Table (1): The clinic-demographic differences between droup I (HCG + IUI) and Group II (IUI alone).

	Group I (150)	Group II (150)	P- value
Age	31.4 ± 3.2	32.1 ± 3.5	> 0.05
Menarche age	11.3 ± 3.2	11.5 ± 3.8	> 0.05
Body mass index (kg/m2)	28.2 ± 3.8	27.6 ± 3.6	> 0.05
Previous gravidity	1 ± 0.8	1 ± 0.6	> 0.05
Type of infertility			
1ry	92	89	> 0.05
2ry	58	61	
Duration of infertility	7.8 ± 3.1	7.6 ± 2.8	> 0.05
Education			
≤High school	98	96	> 0.05
>High school	52	54	
Occupation			
House wife	109	106	> 0.05
Employed/business Woman	41	44	
Number of developing follicles at insemination	2.7±1.6	2.8±1.3	> 0.05
Mean diameter of dominant follicles at insemination	20.2 ± 1.5	19.2± 2.6	> 0.05
Mean endometrial thickness	8.2 ± 1.9	8.3 ± 1.7	> 0.05

* Analysis using independent student's t-test. NS = non-significant

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Table (2) shows a comparison between the two studied groups as regards the biochemical and clinical pregnancy rates

Pregnancy rates	Group I No.	(%)	Group II No. (%)		P
Biochemical pregnancy	41	27.3	32	21.3	< 0.05(sig)
Clinical pregnancy	33	22	24	16	< 0.05(sig)

Table (3): Patient compliance and acceptability during and after HCG flush

Patient compliance	No.	(%)
Mild discomfort	67	44.7
Moderate discomfort	43	28.7
Severe discomfort	17	11.3
No discomfort	23	15.3
Difficult flushing	22	14.7
Acceptability	102	68

Table (4) side effects of HCG flush

	Group I N (%)	Group II N (%)	P- value
Nausea	32 (21.3)	10 (6.7)	< 0.05
Vomiting	15 (10)	3 (2)	< 0.05
Abdominal cramping	56 (37.3)	9 (6)	< 0.05
Vaginal bleding	19 (12.7)	4 (2.7)	< 0.05
No. of patients reporting >1 of the above side effects (n)	34 (22.7)	7 (4.7)	< 0.05

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IV. Discussion

Blastocyst implantation needs an extensive angiogenesis at the feto-maternal interface. HCG can modulate the receptivity of the uterine stromal cells to interleukin-1 by upregulation of its receptor (IL1R) during the implantation phase. This action has an effect on angiogenesis, which is a pathway by which embryonic growth is enhanced [11]. Berndt et al. showed that HCG displayed a strong angiogenic impact through receptor stimulation of transforming growth factor beta (TGF-β) in endothelial cells, which is a key step in placental formation [12, 13]. Litch et al. produced an intrauterine microdialysis device to evaluate paracrine mediators. After the intake of 500 UI of HCG, they reported a considerable inhibition of intrauterine insulin-like growth factor binding protein 1 (IGFBP-1) and the macrophage colony-stimulating factor (M-CSF), while leukemia inhibitory factor (LIF), the vascular endothelial growth factor (VEGF) and the matrix metallopeptidase 9 (MMP-9) were significantly activated. These effects precede the classical endocrine role of the HCG and would be directly participated in the regulation of implantation [14, 15].

In this study, we confirmed the benefit of intrauterine injection of 5000 IU HCG before IUI. The current study was conducted on 300 women presented at infertility clinic of Ain Shams Maternity Hospital, during the period between June 2012 and June 2015. The study included 2 groups of women: group I [n=150]; women who had preovulatory HCG uterine flush then IUI, and group II [n=150]; women who had undergone IUI without preovulatory HCG endometrial flush. There was no statistically significant difference between the two groups concerning the clinic-demographic parameters including mean age, menarche age, BMI, mean gravidity, duration and type of infertility, educational level, occupation, number of developing follicles at time of insemination, mean diameter of dominant ovarian follicle and mean endometrial thickness. The two studied groups were compared as regards the biochemical and clinical pregnancy rates. In group I (test group) the biochemical pregnancy rate was 27.3% and clinical pregnancy rate was 22% while in group II (control group) the biochemical pregnancy rate was 21.3% and clinical pregnancy rate was 16% with statistically significant difference between both groups ($P < 0.05$). These techniques would benefit all of women candidates for IUI/IVF cycles.

Intrauterine injection of HCG before the embryo transfers was done for the first time by Mansour et al. 2011, and it was found a significant improvement in the conception rates of the IVF cycles [16]. These improvements would be explained by different changes that are produced in the endometrium, which is where HCG has an effect on implantation [7-14].

Previous studies had reported that there is a key role for HCG in regulating the inflammatory process and angiogenesis during implantation [12], and an altered uterine receptivity by the induction treatments can be

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overcome by injecting HCG before embryo transfers. Xiao-Yan et al. reported that embryos that have a higher production of HCG in their culture media have a positive relation with the implantation rate [15]. 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 in the endometrium instead of having an autocrine role on human embryos.

As regards the patient compliance and experience of discomfort in cases of group I, the patients experienced mild discomfort in (44.7%), moderate discomfort in (28.7%), severe discomfort in (11.3%), also patient acceptability was (68%). Difficult flushing was present in 14.7% of cases. The development of side effects were evaluated, they were low in general but higher than group II with significal difference between the two groups (nausea was present in 21.3% of cases of group I Vs 6.7% in group II, vomiting in 10% in group I Vs 2% in group II, abdominal cramps 37.3% in group I Vs 6% in group II , vaginal bleeding 12.7 in group I Vs 2.7 in group II). There were no long term complications of the intervention. This was in agreement with other studies which reported that uterine flushing is generally easily tolerated, side effects occurred in 8.8% of women and included moderate-to-severe discomfort (4%), vagal symptoms (3%), nausea (1%) and vomiting (0.5%). Pelvic infections are infrequent and were shown to occur in 0.17% of women [16]. To reduce the infection risk, all participants had to present a negative genitourinary test for chlamydia and gonorrhea before initiating the treatment Moreover, we ruled out the possibility of an ongoing conception by doing a urinary pregnancy test before the intervention.

This study could bring a new alternative for the treatment of unexplained infertility. Uterine flushing can represent a less expensive and well tolerated option. It could represent a simple, minimally invasive and easily available treatment, even in limited resources.

V. Conclusion

These results could indicate a new modality for the management of unexplained infertility. Uterine flushing with HCG could represent a cheap and well tolerated choice. It is a minimally invasive, simple and easily available therap, especially in limited resources.

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