

weeks after embryos transfer followed by ultrasonographic evaluation 2 – 3 weeks later for positive cases. Outcome measures of the study were biochemical as well as clinical pregnancy rate and implantation rate. Biochemical pregnancy was defined as quantitative HCG assay more than 50 mIU 1 ml 2 weeks after embryo transfer. Clinical pregnancy was defined as presence of at least one intrauterine gestational sac with fetal pole and cardiac pulsation on transvaginal sonography scan at 4 – 6 weeks after embryo transfer. Clinical pregnancy rate was number of clinical pregnancy divided by the number of embryo transfer procedures. Implantation rate was number of gestational sacs on transvaginal ultrasonography scan at 4- 6 weeks after embryo transfer divided by number of transferred embryos.

Statistical analysis

All statistical analysis were performed using SPSS for windows version 17.0 (SPSS, Inc ,Chicago , Ill ,USA). Continuous variables were analyzed as mean +standard deviation (SD). Categorical nominal data were expressed as frequency and percentages. One way analysis of variance test (ANOVA) was used to compare between basic characteristics of the three groups. Student t-test, Fisher s exact test were used when appropriate to make a comparison between groups .P <0.05 is considered as significance.

Results

195 women were included in the current study that was carried out during the period of Jan 2012 to Jan 2014 There was no dropped out cases in any step of the study. 15 cases (7.7 %) were cancelled because of risk of ovarian hyperstimulation syndrome (9 cases , 4.6 %) and poor embryos quality (6 cases , 3.1%) . There were as follow, 4 cases in group (1), 6 cases in group (2) and 5 cases in group (3). All variables were expressed as mean and standard deviation. Table (1) shows that there was no significant difference between groups as regard age of the participants , duration of infertility , body mass index (BMI) and base line follicle stimulating hormone (FSH) In addition in comparing base line and peak levels of estradiol in all groups , also , no significant difference was noted .

With separate comparison between each group, endometrial thickness at time of HCG administration and number of oocytes retrieved, were nearly similar with no significant difference. Also , the fertilization rate per retrieved oocyte was 70% , 68% and 71.5% while the number of transferred embryos per cycle were 2.8 , 2.4 and 2.65 with no significant difference , tables, 2- 4 .

As regard the outcome measures , the biochemical and

clinical pregnancy as well as the implantation rates were higher in endometrial scratch group than in the control group (55.9%Vs 37.7% , 45.8% Vs 31.1% and 34.9%Vs 21.9% respectively with significant difference $p < 0.05$).Table (2). More increase , in the previous outcomes , was found when comparing the third group (endometrial scratch plus HCG injection)with the first control group and he figures were as follow 60% Vs 37.7% , 50%Vs 31.1%% and 38.1% Vs 21.9% respectively table (3) . .

In an attempt to declare the beneficial effect of flushing the endometrium by HCG together with endometrial scratch, we made a comparison of the outcomes between the second and third groups. The clinical pregnancy rate increase from 45.8% in second group to 50% in the third group as well as improvement of implantation rate by about 8% (from 34.9% to 38.1%) .However , this improvement was not to the degree to be significantly different table (4) .

Table (1)

Patients characteristics

Variables	Group(1) n = 61	Group(2) N = 59	Group(3) N =60	P
Age (year)	29.45 ±3.7	30.15 ±4.1	28.95 ±3.3	0.353
Duration of infertility (year)	8.4 ±4.1	7.6 ±3.7	8.15 ±4	0.251
BMI (kg / m)	28.80 ±3.1	30.50 ±4.2	29.30 ±3.4	0.235
FSH (mIU/ml	6.32 ±2.85	6.74 ±3.23	7.15 ±2.54	0.548
Basal estradiol	35.86 ±10.56	38.35 ±11.15	37.15 ±10.40	0.634
(pg / ml)	2850.45 ±925	2785.30 ±885	2710.50 ±845	0.215

Table (2)

Study outcome in group 1 & 2.

Variables	Group(1) n = 61	Group(2) N = 59	P
Endometrial thickness on day of HCG (mm)	10.46 ±4.2	10.14 ±4.05	0.527
Number of oocytes retrieved	13.15 ±5.17	12.35 ±4.35	0.714
Fertilization rate per retrieved oocytes	70% ±15.45	68% ±18.10	0.483
Number of transferred embryos per cycle	2.8 ±0.4	2.4 ±0.5	0.265
Biochemical pregnancy rate	23/61 37.7%	33/59 55.9%	0.050
Clinical pregnancy rate	19/61 31.1%	27/59 45.8%	0.045
Implantation rate	32/146 21.9%	44/126 34.9%	0.035

Table (3)

Study outcome in group 1 & 3.

Variables	Group (1) n = 61	Group (2) N = 60	P
Endometrial thickness at day of HCG (mm)	10.46 ±4.2	10.54 ±4.27	0.74
Number of oocytes retrieved	13.15 ±5.17	12.85 ±4.90	0.437
Fertilization rate per retrieved oocyte	70% ±15.45	71.5% 16.15	0.513
Number of transferred embryos per cycle	2.8 ±0.4	2.65 ±0.44	0.445
Biochemical pregnancy rate	23/61 37.7%	36/60 60%	0.035
Clinical pregnancy rate	19/61 31.1%	30/60 50%	0.030
Implantation rate	32/146 21.9%	60/153 38.1%	0.015

Table (4)

Study outcome in group 2 & 3.

Variables	Group (1) n = 59	Group (2) N = 60	P
Endometrial thickness on day of HCG (mm)	10.14 ±4.05	10.54 ±4.27	0.431
Number of oocytes retrieved	12.35 ±4.35	12.85 ±4.90	0.445
Fertilization rate per retrieved oocytes	68% ±18.10	71.5% ±16.5	0.389
Number of transferred embryos per cycle	2.40 ±0.5	2.65 ±0.44	0.183
Biochemical pregnancy rate	33/59 55.9%	36/60 60%	0.214
Clinical pregnancy rate	27/59 45.8%	30/60 50%	0.158
Implantation rate	44/126 34.9%	60/153 38.1%	0.116

Discussion

Implantation is a multifactorial complex process where interaction between different molecular and hormonal pathways take places. In spite of great advances in biotechnology of assisted reproduction, there still be a great discrepancy between embryo transfer rate and the resultant low implantation rate with up to 70% of embryos lost. For better implantation and success of ICSI, it is important to transfer a morphologically good embryo inside uterine cavity with receptive endometrium (18).

HCG is secreted from early embryo before implantation and has appreciative role in further embryonic development. One of key regulator of inflammatory response, endometrial immune tolerance and angiogenesis associated with successful implantation, is embryonic HCG (19, 16). Moreover, it was found

that embryos with higher secretion of HCG have more potentiality for successful implantation (20). Also, local endometrial injury was found to increase the clinical pregnancy and implantation rate. This attractive benefit is not fully understood but may be related to induction of inflammatory response with release of proinflammatory growth factors and cytokines that may play a role in successful implantation (11).

Many researches were designed to evaluate the effectiveness of endometrial injury for improving the endometrial receptivity. Endometrial injury, during the luteal phase of the cycle immediately preceding the ICSI trial, was employed in the present study where significant improvement of the clinical pregnancy (45.8% Vs 31.1%) and implantation rates (34.9% Vs 21.9%) were observed in group (2) in comparison to group (1). These findings were in agreement with the results shown by Zhou L et al (9), with important difference that we included cases with previous implantation failure only. Also, in consistent with our results, Suleyman et al (22), when examined the effect of double endometrial scratch in the immediate preceding luteal phase and in early follicular phase of stimulation cycle, in normoresponder with single embryo transfer, they reported improvement of clinical pregnancy rate (48.2%) in the intervention group more than control group (29%) (p = 0.025). Furthermore, in cases with previous implantation failure, less figures, but still statistically significant improvement was found with clinical pregnancy rate (32.7% Vs 13.7%) and implantation rate (13.07% Vs 7.1%). They employed two endometrial injuries technique at follicular and luteal phases if the immediate preceding cycle (11, 21). On contrary to the previous good results, other author did not find significant difference between the intervention and the control groups in terms of chemical, clinical and implantation rates when offering luteal endometrial scratch (23).

According to the knowledge about the central and well established effect of HCG in regulating implantation, some investigators studied the effect of parenteral HCG administration during luteal phase after frozen-thawed embryo transfer and found no significant difference in clinical pregnancy and implantation rates (24, 25). Moreover, few authors evaluated the effect of flushing endometrial cavity with HCG just before embryo transfer as Mansour et al (17) who restricted their work to fresh first time cycle only and Alvaro S et al (26) who included frozen-thawed embryo as well as previous implantation failure in his study. Both authors found statistically significant improvement in terms of clinical pregnancy and implantation rates, in the intervention and control groups, which were 75% Vs 60% and 41.6% Vs 29.5% in Man-

sour study and 51% clinical pregnancy rate Vs33% in Alvaro S et al study(26) .In the current study, in the third group , with flushing the endometrial cavity with HCG before embryo transfer and preceded endometrial scratch , there were improvement in clinical pregnancy and implantation rates in comparison to first control group(50% Vs31.1 % and 38.1% Vs 21.9% with significant difference $p < 0.05$) . This is in consistent with the results obtained by Mansour et al (17) and Alvaro S et al (26) It import us to know that our study population was restricted only to cases with previous implantation failure and in the same time frozen – thawed embryos were excluded . The improvement in the third group may be explained partially by the effect of endometrial scratch and partially due to intrauterine injection of HCG before embryo transfer To explore if there is significant improving effect of HCG endometrial flushing to a previously scratched endometrium , we made a comparison between the second and the third groups .There were increase in clinical pregnancy rate by about 10%(from 45.8% in group (2) to 50% in group (3) and implantation rate from 34.9% in group (2) to 38.1% in group (3) but , unfortunately , the improvement was not statistically significant .These may be explained by the relatively small number of each group , choice of the transferred embryos depending on morphological assessment only without adding metabolic evaluation as well as exclusion of thawed embryos where intrauterine injection of HCG is thought to give good outcome .Also , both procedures may improve the outcomes through sharing some pathways responsible for implantation .

Conclusion

Combined endometrial scratch (in the luteal phase of the immediate preceding cycle) with HCG endometrial flushing (before embryos transfer) is a simple safe and cost – effective procedure and is associated with improvement of ICSI outcomes. It may be, in future, offered as a routine step in all IVF I ICSI cycles. More trials are needed to reproduce the outcomes in a large scale with miscarriage and live birth rates should be added for outcome measures.

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The role of endometrial scratching and intrauterine injection of HCG for optimizing the outcome of ICSI after implantation failure

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Abstract

Objective: To evaluate the effect of endometrial scratching plus pre transfer flushing of uterine cavity by HCG on clinical pregnancy and implantation rate in cases with previous implantation failure.

Patients & Methods: The study includes 195 infertile ladies with previous implantation failure. They were randomly divided into three equal groups. All groups would offered ICSI cycle employing either long GnRH agonist or the GnRH antagonist protocol according to patient characteristics. In the first group, no further intervention was done and in the second group, endometrial scratch during the luteal phase of the immediate preceding cycle was employed, while in the third group endometrial scratch plus pre transfer intrauterine injection of HCG were done.

Results: All variables were expressed as mean + standard deviation. In all studied groups, there were no significant difference as regard their age, duration of infertility, body mass index and base line follicle stimulating hormone as well as base line and peak level of estradiol (P values > 0.05). The biochemical and the clinical as well as the implantation rates were higher in the endometrial scratch group than in the control group (55.9% Vs 37.7%, 45.8% Vs 31.1% and 34.9% Vs 21.9% respectively with significant difference, P< 0.05). More increase in the same outcome measures were noted when comparing the third group with the first group (60% Vs 37.7%, 50% Vs 31.1% and 38.1 Vs 21.9 % respectively, P values< 0.05). The clinical and implantation rates increase in the third group more than the second group (45.8% Vs 50% and 34.9% Vs 38.1%) with no statistically significant difference, P> 0.05).

Conclusion: We found that adding pretransfer intrauterine injection of HCG to endometrial scratch improves the implantation rate in ICSI cycle.

Key Words: endometrial scratch, intrauterine HCG, ICSI, implantation.

Introduction

Infertility is not only worldwide medical problem but also a psychosocial one as well with incidence about 10 – 15 % (1). Many treatment strategies have been developed, aiming at achieve pregnancy, with In vitro fertilization and Intracytoplasmic sperm injection (IVF / ICSI) came as last step. A large drop is present between embryo transfer and occurrence of pregnancy, with up to 70 % of embryo losses occur at time of implantation, and reducing such high figure represent a great challenge (2). Multiple variables could affect the success of IVF /ICSI, among which is optimum endometrial receptivity which represents the corner stone of successful implantation (3)

Failure of implantation may arise from abnormalities of endometrium, embryo or immune system (4). Needless, Implantation, to progress in its successful pathway with opposition, attachment and invasion, need a certain dialogue between receptive endometrium and a healthy good quality embryo (5, 6). This dialogue is complex and regulated by interaction between hormonal, growth factors, cytokines and certain gene expression (7). Again, implantation, in its basic mechanism, represents an inflammatory reaction of the endometrium to the embryo. Employing such fact,

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many investigators started to induce or improve such inflammatory response by many ways aiming to improve endometrial receptivity and implantation with subsequently increase the success of IVF / ICSI.

For many years, endometrial scratch before IVF/ICSI was employed to improve endometrial receptivity and implantation with controversial results obtained depending on timing of doing endometrial injury (8-11). Recently, a systematic review and meta-analysis demonstrated that endometrial injury / scratch during luteal phase of the cycle preceding IVF- ICSI trial is associated with 70% improvement in clinical pregnancy rate (Relative Risk 3.32, 95% CI 1.72 -3.13) (12). Endometrial injury may act through, firstly, induction of decidualization as shown in animal study (13). Secondly, release of different cytokines and growth factors which are important in process of implantation as, leukemia inhibitory factor, and interleukin-11 and heparin-binding endothelial growth factor (11).

Moreover, the receptivity of the endometrium is considered as a steroid hormone, estrogen as well as progesterone dependent process and is limited to a certain period of time, implantation window, during which a potentially healthy embryo is allowed to adhere and invade (14). Human Chorionic Gonadotropin (HCG) required for pregnancy maintenance is secreted by the early embryo and through its effects upon inflammatory and endothelial cells responsiveness as well as release of angiogenic factors could have early effective role in regulating implantation (15, 16). Based on these important effects of HCG upon implantation, Mansour et al. (17) was the first to report the work with intrauterine administration of HCG with significant improvement of pregnancy rate in IVF / ICSI cycle.

The aim of this study was to evaluate the effect of flushing the endometrial cavity with HCG, before embryo transfer, in association with endometrial injury, done in the immediate preceding cycle, upon clinical pregnancy and implantation rates of ICSI cycles with previous implantation failure.

Patients and Methods

A total of 195 participants with infertility were included in the study. They were recruited from fertility care unit, Mansoura university hospital and private assisted reproduction clinic during the period from Jan 2012 till Jan 2014. The research protocol was approved by research committee of the university. The inclusion criteria were women age under 40 years, body mass index less than 35, no thrombophilia or immunological disorders or under control, no uterine myoma and within normal uterine cavity by hysteroscopic examination and previous IVF-ICSI trial with implantation failure.

Azoospermia and frozen embryo were excluded from the study.

Explanation of the study is offered to all participants and informed written consent is obtained then the participants were randomly allocated into three groups. Randomization was carried out through a computer generated number and a sealed envelope opened by a nurse. All groups would be offered ICSI cycle employing either long GnRH agonist or the GnRH antagonist protocol according to the indication of treatment and patient characteristics. The first group (n=65) with no further intervention was done, women in the second group (n=65) would be subjected to endometrial scratch during luteal phase of the preceding cycle and finally, the third group (n=65) where endometrial scratch was done during luteal phase of the preceding cycle in association with intrauterine injection of HCG just before embryo transfer.

Endometrial scratch, for second and third groups, was done as an office procedure on day 21 – 24 of spontaneous cycle preceding ICSI trial with or without systemic analgesia. Participant laid in lithotomy position with insertion of Cusco speculum then clean ectocervix followed by introduction of Pipelle biopsy catheter (Pipelle de Cornier, Proimed, Neuilly – en – Thelle, France) with gentle endometrial scratch in different directions, rotatory and up and down movements. The routine use of prophylactic antibiotic was not warranted.

After oocytes retrieval, most suitable sperms would be chosen to be injected into most suitable oocytes two to three days later; the embryos were assessed morphologically for quality. In all groups in the third day, one to three grade A1 B quality embryos chosen for transfer under guidance of abdominal ultrasonography with full or partially full urinary bladder. The woman laid in lithotomy position with visualization of cervix using Cusco speculum then cleaning of the portio was done followed by partial suction of cervical mucus using 1 mL syringe then irrigation of the cervix by embryo culture medium. In the first and second groups, soft catheter (Wallace – Smith Medical International Ltd) was loaded by embryos and at mid uterine cavity, the embryos were placed and catheter left in situ for few minutes before withdrawal and then examined. In the third group, employing the procedure described by Mansour et al, 2011 (17), just before embryo transfer, 500 IU of HCG was added to 40 ul tissue culture medium was injected inside uterine cavity using soft catheter which was left in situ for 7 minutes then embryo (s) transfer was (were) done as described before.

Luteal phase support was carried on as scheduled then quantitative serum assessment of HCG was done two