Impact of intrauterine injection of human chorionic gonadotropin Vs intrauterine infusion of autologous platelet rich plasma Vs endometrial scratch on pregnancy rates as management of unexplained recurrent implantation failure (A prospective comparative study)

Doaa Abdelnaby Ragab Refaey Marey, Lamiaa Mohamed El-Ahwal, Dina Gamaleldeen El-Kholy and Adel El-Shahat Elgergawy

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Abstract

Background: Infertility is one of the most common problems among couples worldwide. In vitro fertilization (IVF) is the gold standard treatment for causes of infertility, including tubal obstruction, severe male factor, poor ovarian reserve, and unexplained infertility of long duration. Implantation is an essential physiological phenomenon for pregnancy and IVF success. It is referred to the attachment of the embryo to the endometrial luminal surface of the uterus. The aim of this study is Assessment and evaluation the effect of intrauterine injection of human chorionic gonadotropin before embryo transfer of IVF cycle versus intrauterine infusion of autologous platelet rich plasma versus the endometrial scratch on the clinical pregnancy rates as a management of the cases with unexplained RIF.

Methods: A prospective comparative study was done in Tanta university hospital and private fertility centers.

Group A (30 cases): the cases in this group had been subjected to instillation of the endometrial cavity with HCG. Intra-caviar instillation of HGG was mediated immediately before ET in a dose of HGG (≤ 500 international unit).

Group B (30 cases): the cases were subjected to endometrial scratch or injury.

Group C (30 cases): the participants in this group were treated by intrauterine infusion of autologous platelet rich plasma (PRP).

Results: there was no statistically significant difference between the three studied groups regarding the basic hormonal profile (FSH, LH, E2, TSH, Prolactin and AMH). there was no statistically significant difference between the three studied groups regarding (Number of oocyte retrieval, Number of oocytes fertilized, Number of embryo transfer and Duration of stimulation (days). there was no statistically significant difference among the three studied groups according to the biochemical, clinical, ongoing pregnancy and miscarriage rates. The clinical pregnancy rates in group C using PRP was little higher than group A and B. there was no negligible difference in the miscarriage rate across the three groups. Due to the small sample size, there was no statistically differences, the study needed more time and data to draw firm conclusions

Conclusions: RIF is a highly fertility obstacle so larger studies with great experience in handling and preparation are recommended to reach an evidence based solid conclusion regarding the use of these different modalities in RIF.

Keywords: Intrauterine, human chorionic gonadotropin, platelet rich plasma, endometrial scratch, implantation failure

Introduction

Infertility is one of the most common problems among couples worldwide. In vitro fertilization (IVF) is the gold standard treatment for causes of infertility, including tubal obstruction, severe male factor, poor ovarian reserve, and unexplained infertility of long duration [1].

Implantation is an essential physiological phenomenon for pregnancy and IVF success. It is referred to the attachment of the embryo to the endometrial luminal surface of the uterus [2].

Recurrent implantation failure (RIF) is one of the most challenging problems in assisted
reproductive technology (ART). It is defined as the failure to achieve a pregnancy after transferring high-grade embryos through at least three IVF cycles to the endometrium [3]. Implantation is a multi-factorial process which means that several factors contribute to its success or failure. RIF accounts for 50–70% of unsuccessful pregnancies. There are different causes for RIF: it includes advanced maternal age, the influence of the male factor and sperm quality, embryo quality, subclinical thyroid dysfunction, immunological diseases, disturbed coagulopathy and the various uterine pathologies [4].

The different uterine pathological conditions contribute in the RIF including small sub-mucous fibroid or polyp, intrauterine synechia, chronic endometritis (CE) and occult endometriosis. The incidence of uterine pathology in women undergoing IVF has been reported to be as high as 40% [5, 6]. Occult endometriosis is considered a possible aetiology for RIF as it is associated with decreased integrin expression and increased aromatase expression. CE is correlated with reproductive failure and related mainly to the advanced maternal age and repeated pregnancy loss [7].

Patients undergoing IVF cycle have ongoing all infertility profile for complete evaluation including hormonal profile, semen analysis, thyroid function tests and ultrasonography. For evaluation of the endometrium cavity prior to embryo transfer (ET), hysterosalpingogram (HSG), saline-infusion sonography (SIS), and hysteroscopy are all accepted tools for cavity assessment. While hysteroscopy is widely regarded as the gold standard method [8], Endometrial injury or “scratch”. The idea of endometrial injury to improve implantation is showing benefit in patients with prior failed transfers. The concept is based on expression of an acute inflammatory reaction, followed by repair, resulting in the release of cytokines and multiple GFs known to promote implantation. Optimal timing for positive effects of the procedure was the cycle preceding transfer, with same-cycle scratch possibly detrimental to pregnancy rate (9). Aim of the work is Assessment and evaluation the effect of intrauterine injection of human chorionic gonadotropin before embryo transfer of IVF cycle versus intrauterine infusion of autologous platelet rich plasma versus the endometrial scratch on the clinical pregnancy rates as a management of the cases with unexplained RIF.

Methods
A prospective comparative study was done in Tanta university hospital and private fertility centers. Patients: The study included a total of 90 females suffered from RIF after several IVF/ICSI cycles during the period of October 2020 till October 2022.

Inclusion criteria
Age under 35 years old.
Had at least two implantation failures following IVF cycle. Good ovarian reserve (Anti-Mullerian Hormone [AMH] > 1 ng/ml)

Exclusion criteria
Ovarian disorders: PCOS, endometrioma or ovarian cyst.
Anatomical uterine pathology (uterine septum, polyp, arcuate uterus or bicornate uterus).
Common causes of implantation failure as diabetes mellitus, hypertension, autoimmune diseases, suspected or diagnosed hydrosalpinx, congenital and acquired thrombophilia, thyroid dysfunctions and female genital tract infection.

Methods: All cases had been subjected to the following: Full medical history including hematological, cardiac, autoimmune etc.

Full general and abdominal examination
Complete infertility profile including semen analysis, hormonal profile, thyroid function tests, hysterosalpingography and ultrasonography.
Routine lab investigation (CBC, PT, PTT).
Investigation of diagnosed autoimmune disorders and anti-phospholipid antibody syndrome.

Group A (30 cases): the cases in this group had been subjected to instillation of the endometrial cavity with HCG. Intracavitary instillation of HGG was mediated immediately before ET in a dose of HGG (≤ 500 international unit). A HGG ampoule included 5000 international units, it was estimated for 500 international unit via the ET media (1 mL of tissue culture media) in the laboratory.

Group B (30 cases): the cases were subjected to endometrial scratch or injury. The endometrial scratching was done once at day 21 of menstrual cycle in the cycle prior to ET. Anterior and posterior walls of endometrium will be scratched gently by Novak curette inserted through the cervical os.

Group C (30 cases): the participants in this group were treated by intrauterine infusion of autologous platelet rich plasma (PRP). Preparation of PRP was an office procedure that involved withdrawal of venous blood, preparation of the PRP, and then injection of it intrauterine through ET catheter 48h before ET under ultrasound guidance. All the patients in the three groups were prepared for embryo transfer by receiving 2 mg Estradiol Valerate (Cycloprogenova, Bayer Schering Pharma”the white tablets”) daily from day 2 of the menstrual flow with addition of 100 mg Progestrone (Prontogest daily IM injection from day of embryo transfer till pregnancy test becomes positive and continued till 12 weeks of gestation.

PRP was ready for injection. Nearly 30 ml of venous blood yields 3-5 ml of PRP which contained platelets at about 4–5 times higher in concentration than circulating blood. About 0.5-1ml of PRP was infused intrauterine through the ET catheter. The syringe containing the PRP was connected to ET catheter and the PRP was infused. Then the syringe filled with air was used to push in the remaining PRP. Then the air bubbles were confirmed by ultrasonography.

Procedure of the endometrial scratch
The scratch was performed once in the mid-luteal phase (at day 21) of a natural cycle or in a hormonal controlled cycle. The patient was put in lithotomy position. The cervix had been exposed by a Casco speculum. Cleaning the vagina and cervix was done by sterile saline.

Embryo transfer (ET): The patient was put in the lithotomy position, and the cervix was ~28~
visualized using Cusco’s speculum. The cervical mucous was wiped out using a sterile piece of gauze.
A dummy ET was performed using a rigid but malleable catheter (Cook IVF).
After the catheter had passed the internal cervical os, embryos were loaded into the ET catheter and were transferred into the uterine cavity under guidance of ultrasonography.
For approximately 5 minutes after the ET, the vaginal speculum was left in place, slightly pressing on the portio-vaginalis of the cervix, then it was removed.

**Statistical analysis**

SPSS version 27 (IBM©, Chicago, IL, USA) for Windows was utilized to evaluate the data. Quantitative data was reported as mean SD or median (range) according to normality, whereas qualitative data was expressed as number and percentage. According to the nature of the data, the relevant statistical tests were employed and were judged statistically significant (P value ≤ 0.05).

**Results**

<table>
<thead>
<tr>
<th>Table 1: Demographic and clinical characters of the studied groups according to (the age, BMI, duration and type of infertility)</th>
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<tbody>
<tr>
<td>Groups</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td></td>
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<tr>
<td>BMI (Kg/m2)</td>
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<td>Duration of infertility (years)</td>
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<tr>
<td>Type of infertility N (%)</td>
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</table>

Table 1: there was no statistically significant difference in demographic data of patients. Table 1 shows the demographic data of the studied groups in which there was no statistically significant difference between the three studied groups regarding demographic data of the patients (age, BMI, and duration of infertility).

<table>
<thead>
<tr>
<th>Table 2: The basic hormonal profile (FSH, LH, E2, TSH, Prolactin and AMH)</th>
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<tbody>
<tr>
<td>Groups</td>
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<tr>
<td>AMH (ng/ml)</td>
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<tr>
<td></td>
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<tr>
<td>FSH (mIU/ml)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
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<tr>
<td></td>
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<tr>
<td>E2 (Basal) (pg/ml)</td>
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<tr>
<td></td>
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<tr>
<td>TSH (mIU/ml)</td>
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<tr>
<td>Prolactin (ng/ml)</td>
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</table>

Table 2 shows that there was no statistically significant difference between the three studied groups regarding the basic hormonal profile (FSH, LH, E2, TSH, Prolactin and AMH).

<table>
<thead>
<tr>
<th>Table 3: Parameters used for follow up of the patient during the duration of induction.</th>
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<tbody>
<tr>
<td>Groups</td>
</tr>
<tr>
<td>Antral Follicular Count (AFC)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Number of Follicles &gt;16 mm during induction (for both ovaries)</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Endometrial thickness at day of HGG triggering (mm)</td>
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</table>

Table 3 showing different parameters evaluating the patient during the period of ovulation induction including AFC, number of follicles and endometrial thickness. There was no statistically significant difference between the three studied groups regarding (AFC), Number of Follicles >16mm during induction and endometrial thickness at day of HGG triggering (mm)).

Table (4): there was no statistically significant difference between the three studied groups regarding (Number of oocyte retrieval, Number of oocytes fertilized, Number of embryo transfer and Duration of stimulation (days)).

Table 4 showing there was no statistically significant difference between the three studied groups regarding (Number of oocyte retrieval, Number of oocytes fertilized, Number of embryo transfer and Duration of stimulation (days)).
Table 4: Parameters of IVF/ET cycle.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group A (n = 30)</th>
<th>Group B (n = 30)</th>
<th>Group C (n = 30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of oocyte retrieval</td>
<td>Range</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-8</td>
<td>7.17±0.83</td>
<td>7.43±0.5</td>
<td>7.17±0.95</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>7.17±0.83</td>
<td>7.43±0.5</td>
<td>7.17±0.95</td>
</tr>
<tr>
<td>Number of oocytes fertilized</td>
<td>Range</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
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<tr>
<td></td>
<td>5-7</td>
<td>6.1±0.99</td>
<td>6.37±0.85</td>
<td>6.2±1.1</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>6.1±0.99</td>
<td>6.37±0.85</td>
<td>6.2±1.1</td>
</tr>
<tr>
<td>Number of embryo transfer</td>
<td>Range</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
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<tr>
<td></td>
<td>2-5</td>
<td>2.03±0.18</td>
<td>1.87±0.35</td>
<td>1.87±0.35</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>2.03±0.18</td>
<td>1.87±0.35</td>
<td>1.87±0.35</td>
</tr>
<tr>
<td>Duration of stimulation (days)</td>
<td>Range</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-13</td>
<td>11.37±1.16</td>
<td>11.27±1.17</td>
<td>11.47±1.11</td>
</tr>
</tbody>
</table>

Table 5: Cumulative pregnancy rates in the three studied groups (Biochemical, clinical and ongoing pregnancy)

Table 5: There was no statistically significant difference among the three studied groups as regard biochemical, clinical, ongoing and miscarriage rates.

Table 5 showing there was no statistically significant difference among the three studied groups according to the biochemical, clinical, ongoing pregnancy and miscarriage rates.

The clinical pregnancy rates in group C using PRP was little higher than group A and B. There was negligible difference in the miscarriage rate across the three groups. Due to the small sample size, there was no statistically differences, the study needed more time and data to draw firm conclusions.

Discussion

In our study, the demographic data of the selected women were convergent regarding age and BMI with no statistically significant difference between the three studied groups. The age ranged between (27–35) years old with mean (31.9, 30.9, 32.5) according to the groups A, B and C. This excluded the age factor as a cause of unexplained implantation failure as increasing age of the female partner is influential factor in the likelihood of pregnancy. Regarding BMI of the different studied groups ranged between (21 – 38) kg/m².

These findings came in agreement with Davar et al., who investigated 94 poorly responding patients and found that there was no significant difference in mean age, BMI similarly with Schimbieri et al. [10, 11].

This came in line with Safarian et al., who investigated 120 RIF women and found there was no significant difference between the studied groups as regard endometrial thickness [12].

This came in line with Ershadi et al., who showed that there was no significant difference between the studied groups as regard endometrial thickness [13].

Also, Xuemei Liu et al., (2019) published prospective study to evaluate the impact of intruterine administration of HGG on pregnancy outcomes in FET cycles of patients with RIF. The cases study received an infusion of 500 IU of HGG diluted in normal saline as the same dose in our study. But they used large group of the cases (105) compared to ours (30 cases). They inserted the dose 3 days before the embryo transfer different from our study, we inserted the dose in the same day of embryo transfer. Their results showed the clinical pregnancy rate, implantation rate and live birth rate were statistically higher in the hCG-treated group than the control group (37.5% versus 25.17%, 29.19% versus 19.4%, 25.97% versus 17.22%).

However, there were no differences in abortion rate (22.81% versus 26.32%). The results in our study were different, the biochemical pregnancy rate was (30%), the clinical pregnancy rate was (23.3%) and the OPR was (20%) while the MR was (3.33%) [14].

However, a prospective clinical research assessing the effect of the B HGG injection on the incidence of clinical pregnancies was reported by Santibaez et al., (2014). Similar to our research, they employed B-HGG at a dosage of 500 IU, but they applied it to a much larger sample size (101). The rate of clinical pregnancies in this study was 50.4%, significantly higher than the rate of clinical pregnancies in our study, which was reported by Santibaez et al., (2015) [14].

However, our study employed 101 cases study received an infusion of 500 IU of HGG diluted in normal saline as the same dose in our study. But they used large group of the cases (105) compared to ours (30 cases). They inserted the dose 3 days before the embryo transfer different from our study, we inserted the dose in the same day of embryo transfer. Their results showed the clinical pregnancy rate, implantation rate and live birth rate were statistically higher in the hCG-treated group than the control group (37.5% versus 25.17%, 29.19% versus 19.4%, 25.97% versus 17.22%).

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Additionally, Pinxiu Huang et al., (2017) published a prospective analysis of patients undergoing FET who received an injection of HGG 1000IU compared with ours 500IU, also the participants were (225) which was high number compared with our study (30 cases). They injected the HGG 3 days before the embryo transfer different from our study the was injected in the same day of the embryo transfer. Comparing their results on
the cases of RIF to ours, the biochemical pregnancy rates were (49.02% vs 30%), and the CPR (48.05% vs 23.3%) (165). Zhihui Xu et al., (2019) reported a special study using injection of HGG intrauterine aiming to improving the pregnancy rate but it was mainly on the cases of endometriosis. In their study the HGG infusion group were more likely to achieve a higher pregnancy rate (64.4% versus 47.4%) and clinical pregnancy rate (57.8% versus 39.3%) compared with those in the control group. It was more likely as our study; the case group was 33 cases like our group 30 cases but with different results [17].

Criciuas L et al., (2018) reviewed an article evaluated 17 studies explaining intrauterine administration of HGG using more than 500IU in some groups and less than 500IU in other groups for sub-fertile women undergoing assisted reproduction. They evaluated 4751 women with either natural or synthetic hormone which was administered at variable doses at different times before ET. The clinical pregnancy rate was 49%, using hCG < 500 IU and 27% using of hCG ≥ 500 IU. The sample and the results of that study was far from ours [18].

Nastri et al. also included data from a total of 37 studies (8786 women) into their study. In most cases endometrial injury was performed by pipelle biopsy in the luteal phase prior to starting an IVF cycle. This was the same timing as our study but it was done by Novak curate. They suggested that according to the clinical pregnancy, if the chance of clinical pregnancy from IVF is normally 32%, then the chance when using endometrial injury before IVF is between 31% and 37%. Endometrial injury probably results little to no difference in chance of miscarriage [19].

Also, Barash et al., (2003) reported an enhanced implantation rate after local damage by endometrial biopsy in the cycle prior to IVF therapy in a prospective analysis of 130 individuals who had not conceived after one or more IVF cycles. They enrolled 45 cases compared to ours 30 cases. Comparing the results there were some differences, their chemical pregnancy rate was 27.7%, clinical pregnancy rate was 66.7% and 20% MR. In our study the results were different 30%, 23.3% and 3.33% respectively [20].

Also 2014, L. Polanski et al., published a prospective study about how the endometrial biopsy prior to assisted reproductive techniques didn't improve treatment outcome in unselected patient. The study included 76 cases in comparing with our study 30 cases. As our study there was no differences in age, BMI, cause of infertility, number of previous live births, ovarian reserve, embryo quality, or day of transfer were observed. There were no significant differences in CPR were observed in the rates of biochemical pregnancy or first trimester miscarriage [21].

Vitagliano A et al., (2018) reported a systemic review about effect of endometrial scratch injury on women with one or more previous failed embryo transfers which was the same aim of work of our study. They compared between groups with endometrial scratch injury and another placebo but the study included a large number of cases rather than our study which included a small group. In our study we did the endometrial scratch in the day 21 of menstrual cycle in the cycle prior to embryo transfer also in their study all patients received the scratch during the menstrual cycle preceding the ET but with some differences. In five studies endometrial injury was performed twice (during the luteal phase or once during the follicular phase and once during the luteal phase and in the remaining studies it was performed once or during the follicular phase). Comparing the results there also were some differences, they found higher clinical PR and also MR (both) 95% compared to ours 20% and 3.33% [22].

implantation failure, there were no significant differences in their groups regarding duration, dose of gonadotrophin used in ovarian stimulation,umber of retrieved oocytes, number of good quality embryos and in total transferred embryos. They infused 1ml of PRP on the 11th day of menstrual cycle and another one on day 13th on large number of cases (110) which differed from our study as we infused it 48 before embryo transfer on of 30 cases. They also achieved different results, the biochemical and clinical were 15.9% and 10.9% compared with ours 36.5% and 33.3%. They also concluded that in spite of the infused PRP improved the endometrial thickness but didn’t reach the clinical significance [23].

Mehrâza et al., published a retrospective cohort study included 123 patients with history of more than two repeated failed embryo transfers. They compared using intrauterine infusion of PRP (n=67) and systemic administration of GCSF (n=56). In the PRP group, the endometrial preparation was done by Estradiol valerate and Progesterone was started when a triple-line endometrial pattern appeared, the intrauterine infusing of PRP was done 48 hours before the frozen embryo transfer, all were as our study. They found that the biochemical and clinical pregnancy rate was 43.3% and 40.3% compared with ours 36.5% and 33.3% [24].

Haiyu D et al., published a systematic review and meta-analysis about the effect of intrauterine infusion of platelet-rich plasma for women with recurrent implantation failure. Data was extracted from ten different studies (six randomized controlled trials and four cohort studies) involving 1555 patients. They used a dose of PRP, which was infused into the uterine cavity was about 0.5ml and infused before embryo transfer. The data imply PRP has potential to improve pregnancy outcomes in women with RIF, suggesting a promising role in assisted reproductive technology [25].

Another systematic metaanalysis study was published showing value of platelet-rich plasma in women with previous implantation failure. The included study infused the PRP intrauterine before the embryo transfer with possibility of repetition when endometrium didn't reach the aimed thickness which didn't agree with ours as we infused it only one time 48 h before ET. They concluded much promising results as regard biochemical, clinical even live birth rate with negligible effect on miscarriage rate. We concluded better pregnancy rate as regard using PRP in spite of non-statistically differences between the groups [26].

Conclusions
RIF is a highly fertility obstacle so larger studies with great experience in handling and preparation are recommended to reach an evidence based solid conclusion regarding the use of these different modalities in RIF.

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Declarations
Funding: there is no funding

Conflict of interest: Nil

Ethical approval: The study was approved from the ethics committee of Faculty of Medicine, Tanta University.

References


