ORIGINAL ARTICLE

DOES ENDOMETRIAL INJURY IMPROVE THE OUTCOMES IN PATIENTS WITH RECURRENT IMPLANTATION FAILURE?

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Abstract

Recurrent implantation failure refers to failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years. We present this retrospective study, in which we proposed local endometrial injury for the management of the uterine factors in the case of 30 couples with recurrent implantation failure. Our conclusion was that local endometrial injury improved the clinical pregnancy rate for these couples, especially when the study population met specific criteria.

Keywords*: recurrent implantation failure, IVF, endometrial injury, scratching*

Introduction

The implantation of the embryo into the endometrium is considered successful when we have ultrasonographic evidence of intrauterine pregnancy. When we perform the ultrasonography for confirmation of the pregnancy it is best to find an intrauterine gestational sac, with a yolk sac and an embryo with cardiac activity, but we have to find at least an intrauterine gestational sac. Therefore, failure of implantation means failure to achive a ultrasonographic evidence of intrauterine gestational sac[1].

The terms implantation failure and in vitro fertilization (IVF) failure are distinct and must not be confused one with another. The IVF failure may be caused by poor ovarian response, cycle cancellation, fertilization failure, implantation failure or miscarriage after ultrasound confirmation of pregnancy. So the implantation failure is just one of the many reasons of IVF failure[1].

The first definition of RIF comes from 1995, when Coulam et al defined it as the failure in achieving a pregnancy after the transfer of more than 12 embryos in multiple cycles. The definition changed along the years, the same as practice of IVF did. In 2003, Stern et al defined RIF as the failure to achieve a pregnancy after the transfer of more than 10 embryos; Tan et al emphasized it takes the transfer of more than 10 good-quality embryos in 2 to 6 IVF cycles to diagnose RIF. The next to define RIF were Margalioth et al in 2006, as the failure of achieving a pregnancy after more or equal to 3 transfers of good-quality embryos.

Coughlan et al gave the latest definition of RIF in 2014: failure to achieve a clinical pregnancy after transfer of at least 4 good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years[1].

Successful implantation not only requires a receptive endometrium, but also a normal and functional embryo. A synchronization between maternal and embryonic tissues is also necessary. According with the statement above, RIF has two main groups of causes: gamete/embryo factors and uterine factors[1]. This paper addresses to the second group.

RIF is a multifactorial process and besides these two main groups, we can take into account other causes, like hydrosalpinges, thrombophilias, maternal age and immunological factors.

The most important embryo/gamete factors are: poor-quality oocyte, poor-quality sperm, parental chromosomal anomalies, suboptimal embryotransfer technique, suboptimal culture conditions and suboptimal embryo quality. The uterine factors can be divided into congenital uterine anomalies and acquired intracavity conditions (the most frequent acquired conditions are submucous fibroids, endometrial polyps, intrautherine adhesions and adenomyosis[1]).

We can divide the most important investigations used for RIF in investigations for endometrial causes and investigations used for embryologic factors. Among the first group we can count: hysteroscopy for intrauterine pathology, ultrasonography or MRI for structural uterine anomalies, hysterosalpingo-graphy or sonohysterosalpingography for hydrosalpinges, the hormone profile (to rule out endometrial defects secondary to endocrine diseases), endometrial biopsy, blood tests for thrombophilias and antiphospholipid antibodies. The investigations of embryologic factors include: ovarian reserve tests (basal FSH, Anti-Mullerian Hormone (AMH), Antral Follicle Count (AFC)), sperm DNA fragmentation tests (Sperm Chromatin Dispersion Test (SCD), DNA Fragmentation Index (DFI test)), genetic karyotype, and if a structural anomaly is detected, Preimplantation Genetic Testing (PGT).

Elevated basal serum FSH levels were the first biochemical marker of ovarian reserve testing ever employed and they are still used. Values over 10-20 IU/L are associated with diminished ovarian reserve, but the test is not predictive of failure to conceive [17].

AMH is responsible for the regression of the female reproductive organs in male fetuses in utero, and it is a strong inhibitor of ovarian follicle development from primordial to primary follicle stage. AMH is produced by the granulosa cells of small growing follicles that have already been recruited for dominance, but have not yet been selected. The development of AMH as a biomarker for ovarian reserve stands upon the fact that AMH presents less intravariability across menstrual cycles, and although decreased serum AMH levels have been reported in the luteal phase, these fluctuations appear to be noncyclic and not significant. So, AMH can be tested at any time point of the cycle phase [18].

AFC represents the sum of small antral follicles (2-10mm) observed by transvaginal ultrasound in the early follicular phase. It is often employed because it has good inter-cycle and inter-observer reliability. AFC is generally considered to be the best predictor of ocyte yield [19].

Regarding the genetic testing, it has improved along the last years, going from the older technique Fluorescence in Situ Hybridization (FISH) that analyses just a few chromosomes, through array Comparative Genomic Hybridization (aCGH) that analyses all chromosomes, to the new technique of next generation Sequencing (NGS). The "gold standard" in preimplantation genetic testing is the biopsy of blastocysts, their cryopreservation through vitrification and the analysis of a few cells from every embryo through NGS. To avoid the errors and to reflect the limits of genetic testing, the international terminology has been recently changed. So, the terms PGS (Preimplantation Genetic Screening) and PGD (Preimplantation Genetic Diagnosis) have been replaced with PGT-A (Preimplantation Genetic Testing for Aneuploidy) and PGT-M (Preimplantation Genetic Testing for Monogenic/Single Gene Defects).

The endometrial receptivity is dependent on a number of stimuli, including mechanical

stimuli. These ones may improve endometrial receptivity. Barash et al, 2003, explored for the first time the effect of local injury of the endometrium in the cycle preceding IVF treatment and whether it increases the success rate of implantation or not. Barash et al designed a prospective study involving women who failed to conceive after one or more IVF treatment cycles. The endometrial injury was performed immediately before the IVF treatment cycle, on days 8, 12, 21 and 26 of the cycle. They concluded that the treatment doubled the rates of implantation, clinical pregnancy and live births, compared with control subjects who didn't have endometrial biopsies[2].

Subsequently, there have been several studies which examined the benefit of endometrial biopsy or endometrial injury on IVF outcome in women who have repeated failures; the interventions were performed in the early proliferative phase[3,4] , in the early proliferative and luteal phases[2,5], or in the luteal phase[16], for once[1,7], twice[8] or four times[2]. The conclusion of these studies was an injury to the endometrium was of benefit in women with RIF, with the condition it is carried out approximately 7 days prior to the onset of menstruation, immediately before the start of ovarian stimulation for IVF treatment[1]. Performing an endometrial injury on the day of the ovarian punction day significantly reduced the IVF outcomes[6]; because the endometrium requires at least 2 weeks to obtain complete repair after mechanical injury[9].

Many theories regarding the mechanism in which the implantation rate is improved by these procedure have been elaborated: inducing decidualization of the endometrium[10]; modulating the expression of a variety of genes required for implantation[11]; inducing synchronicity of endometrium and embryo[10]. Endometrial healing following injury is associated with a significant increase in the secretion of interleukins, cytokines, growth factors, and macrophages and dendritic cells, all of which are beneficial to embryo implantation[10,12].

The endometrial injury or scratch can be performed by using endometrial biopsy instruments. If it is performed with a pipelle sampler, after the sampler is introduced in the uterine cavity, the inner shaft is withdrawn to create a negative suction force, and the pipelle sampler is gradually rotated as it is moved up and down the endometrial cavity several times to produce the 'scratching' action[1]. The endometrial injury may also be carried out at the time of hysteroscopy[1].

The experimental question of this study was: does local endometrial injury improve the chances of a livebirth in a new IVF cycle for couples with RIF and which category of patients will benefit most from this procedure?

Materials and methods

Study design This retrospective study analyzed 30 fresh autologous embryo transfers performed at Gynera Fertility Center in Bucharest during the period 2016-2017.

Patients We enrolled a number of 30 couples with RIF who underwent a fresh autologous blastocyst or cleavage-stage embryo transfer (ET) cycle. The quality of the endometrium and the uterine cavity were assesed by transvaginal ultrasound and hysteroscopy.

We excluded the frozen-thaw ET cycles and donor oocyte cycles. Other exclusion criteria were: body mass index over 30 (because of the effect of obesity on the embryos, analyzed by many recent studies); patients with autoimmune diseases like systemic lupus erythematosus, miastenia gravis, Basedow or Hashimoto thyroiditis, rheumatic arthritis (because of possible immunologic impairment of the endometrium); age over 40 years (because the latest definition of RIF given by Coughlan includes only women under the age of 40 years); any disease (allergy, rash or other dermatological disease) thet led to administration of corticosteroids after performing the endometrial scratching. We also excluded patients with anatomical abnormalities of the uterus, due to congenital abnormalities of the mullerian ducts, that lead to infertility, recurrent pregnancy los and poor pregnancy outcome: hypoplasia or agenesis of the uterus, unicornuate uterus, bicornuate uterus, septate uterus, didelphys uterus, arcuate uterus and diethylstilbestrol-associated anomaly (T-shape uterus).

Among the 30 couples, we encountered all the major causes of infertility: tubal and peritoneal pathology (tubal occlusion and adnexal adhesions), ovulatory disfunction (oligoovulation or anovulation), endometriosis, low ovarian reserve, male factor, unexplained infertility.

All patients had a diagnostic hysteroscopy performed at least one cycle before the endometrial biopsy. We used a 3 mm flexible hysteroscope, with no general anesthesia, as an outpatient. We didn't administer any antibiotics. We divided the patients into 2 groups: one group of 15 RIF patients for which we used local endometrial injury (the L group) and another group of 15 RIF patients as a control group (the C group).

There were no differences between the two groups regarding the age of the patients and the distribution of the infertility causes.

In the L group we performed endometrial scratching with endometrial biopsy instruments (pipelle samplers) in the midluteal phase in some cases (days 20-21 of the cycle) and in the late luteal phase (days 24-25 of the cycle) for other cases. The scratching was done systematically on the uterine fundus and on the four uterine walls. We didn't administer an antibiotics after the procedure. We chose not to perform local endometrial injury through hysteroscopy because the lack of standardization of this procedure.

Ovarian Stimulation Protocol We used mixed FSH/LH protocols. We used long protocol with GnRH-agonists in the presence of endometriosis or low ovarian reserve, counting 9 women. We initiated the administration of the agonist between days 18 and 21 of the previous cycle for the GnRH-agonist cycles. Because of the initial flare-up effect of the GnRH-agonist, we checked when the menses appeared the basal value of the estradiol (it has to be under 50 pg/ml) and the ultrasonographic aspect of the ovaries, in order to exclude the existence of ovarian cysts or already recruited follicles (in the last case we maintained the administration of GnRH-agonist for a longer period, until the pituitary down-regulation appeared).

For most of the couples (21 women) we used short protocol with GnRH-antagonists, because it has multiple advantages: shorter stimulation period with reduced costs and higher compliance of the patients, the possibility of triggering with GnRH-agonists and controlling in this way ovarian hyperstimulation syndrome. We started the administration of the antagonist when the lead follicle was 13-14 mm in size or when the estradiol level was above 300-350 pg/ml.

We didn't use in our study any couple for which we applied the "freeze-all protocol". The freeze-all concept implies on one hand removing the biggest threat in IVF, meaning OHSS (Ovarian Hyperstimulation Syndrome), and on the other hand, freezing all embryos in order to transfer them in a subsequent IVF cycle. OHSS is a iatrogenic complication of assisted reproduction techniques, which is caused by the administration of human chorionic gonadotrophin (hCG) to induce ovulation. This leads to ovarian enlargement and fluid shift from intravascular space to the third compartment due to an increase in capillary permeability, which may lead to hypovolemia, edema, ascites, thrombosis, and in the end even death. The freeze-all protocol uses another substance to trigger ovulation, a GnRH-agonist, and not hCG, and therefore, it avoids OHSS. The next step implies vitrification of the embryos and performing the embryotransfer in the next cycle; so the pregnancy is postponed for a few weeks, meaning the production of hCG is postponed, avoiding again OHSS.

We excluded the frozen-thaw ET cycles and this implied excluding the "freeze-all protocol" couples. We enrolled only couples which underwent a short protocol with GnRH antagonists and a fresh embryotransfer.

For most of the couples (20 patients) we used a constant dose for the stimulation treatment until the patients reached the necessary follicle dimensions and estradiol levels, but we also used step-up dosing regimens (8 patients) or step-down dosing regimens (2 patients).

We used recombinant FSH or human menopausal gonadotropin for ovarian stimulation. We triggered the final oocyte maturation with recombinant hCG for all 9 GnRH-agonist cycles; for the GnRH-antagonist cycles we used double triggering (recombinant hCG and GnRH-agonist) for 17 patients and just GnRH-agonist for 4 patients.

The ovarian punction was performed under general intravenous anesthesia and ultrasound-guided.

The embryologist used for insemination conventional IVF in 12 cases, intracytoplasmic sperm injection (ICSI) in 13 cases and both in 5 cases, as clinically indicated.

Embryo transfer We performed only ultrasound-guided ET with cleavage-stage embryos for 8 patients and with blastocysts for 22 patients.

We used only good-quality embryos, according to the Istanbul consensus workshop on embryo assessment in 2011. In the case of cleavage-stage embryos, that meant using an optimal day-2 embryo with 4 equally sized mononucleated blastomeres, with less than 10% fragmentation, or using an optimal day-3 embryo with 8 equally sized mononucleated blastomeres, wiyh less than 10% fragmentation. In the case of blastocysts, the optimal embryo is a fully expanded blastocyst going to hatched blastocyst; the inner cell mass is prominent and consists of many cells, which are compacted and tightly adhered together; the trophectoderm has many cells forming a cohesive epithelium.

We used Sydney IVF catheters, we didn't use any anesthesia for this procedure. For the luteal support we administered 600-800 mg progesterone daily.

We assessed the serum hCG levels 2 weeks after the trigger injection, and confirmed pregnancy by ultrasonography after another 2 weeks in all pregnant patients.

Results

Up to date, there have been several systematic reviews and meta-analyses regarding the potential benefits of endometrial injury on RIF. The first one was by Potdar et al, 2012, and they concluded that the clinical pregnancy rates were twice as high with biopsy/scratch as compared to hysteroscopy, and that inducing local endometrial injury(LEI) in the preceding cycle of ovarian stimulation will be 70% more likely to result in a clinical pregnancy compared to no intervention[13].

The second meta-analysis was performed by El-Toukhy et al in 2012, and it showed that clinical pregnancy rate was significantly improved after local endometrial injury in both the randomized and non-randomized studies.

The third systematic analysis on the endometrial injury in women undergoing assisted reproductive techniques (ART) is the Cochrane Database in 2015, and their conclusion was: moderate quality evidence indicates that, if it is done between day 7 of previous cycle and day 7 of embryo transfer cycle, the endometrial scratching is associated with improvement in live birth rate and clinical pregnancy rate in women with more than or equal to 2 previous unsuccessful embryo transfers[15].

The fourth meta-analysis, by Coughlan et al 2015, tried to determine the effectiveness of endometrial injury before embryo transfer in women undergoing ART, and showed that the published evidence to date suggested that LEI in the cycle immediately preceding the embryo transfer cycle improved clinical pregnancy rates in those with at least 1 previous unsuccessful embryo transfer.

For our study, figure 1 shows that in the L group, 9 biopsies were performed during the midluteal phase (days 20-21) and 6 biopsies were performed in the late luteal phase (days 24-25). All endometrial biopsies were performed just once.

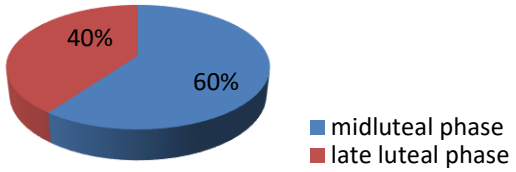

Figure 1 - The timing of the endometrial biopsy in the L group

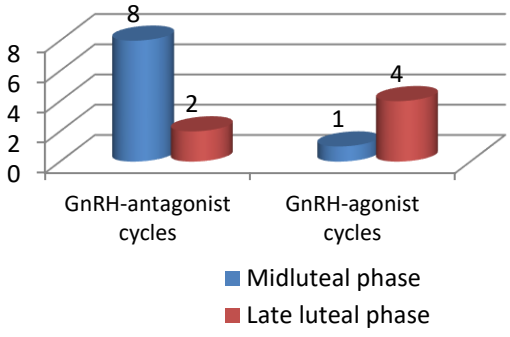

Figure 2 - The timing of the endometrial biopsy in the GnRH-antagonist/agonist cycles

For the patients with GnRH antagonists cycles the endometrial scratching was performed more often in the midluteal phase, while for the patients with GnRH-agonist cycles it was performed just in one case in the midluteal phase (Figure 2).

We looked for the following parameters: biochemical pregnancy (detectable serum human chorionic gonadotropin 2 weeks after the trigger injection), clinical pregnancy (ultrasonography with intrauterine gestational sac +/- embryo with cardiac activity) and live birth (birth of a live infant after more than 28 weeks of gestation) (Figure 3).

The secondary outcome were: the rate of miscarriage, the rate of multiple pregnancies, the rate of abnormal placentation, the rate of pregnancy complication as gestational hypertension/ preeclampsia, the patient tolerance to pain during the endometrial scratching based on the visual score 1-10 and the post procedural metrorrhagia rate.

From the total of 5 patients that presented an ultrasound suspicion of adenomyosis, 2 were from the L group and 3 from the C group. None of those patients obtained a pregnancy after IVF, therefore we hypothesized the negative influence that ademyosis has on infertility. Further studies are needed to confirm this hypothesis.

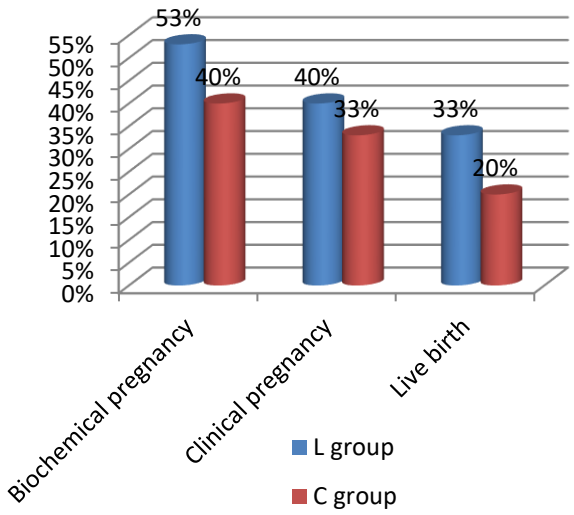

Figure 3 - The outcomes for the groups C and L

There were no significant differences between the age of the patients, the cause of infertility, BMI, AFC or hormonal profile in the two groups. Also, there were no differences in regard to the ovarian stimulation protocol or to the ovulation trigger.

In the L group, the mean number of oocytes retrieved was of 10,4, whereas in the C group we retrieved a mean number of 8,2 oocytes.

The mean number of obtained cleavage-stage embryos was 8,4 in the L group and 6,9 for the C group. Regarding the blastulation rate, we obtained a mean number of 5,3 blastocysts in the L group and 3,9 blastocysts in the C group.

Biochemical pregnancy was higher in the L group than in the C group (8 cases compared to 6 cases); clinical pregnancy was higher in the L group (6 cases) than in the C group (5 cases); live birth was also higher in the L group than in the C group (5 cases compared to 3 cases); because of the small number of subjects, the study lacks statistical significance.

In the L group, the difference between outcome of the IVF cycle in the GnRH-antagonist cycles and the GnRH-agonist cycles had no statistical significance.

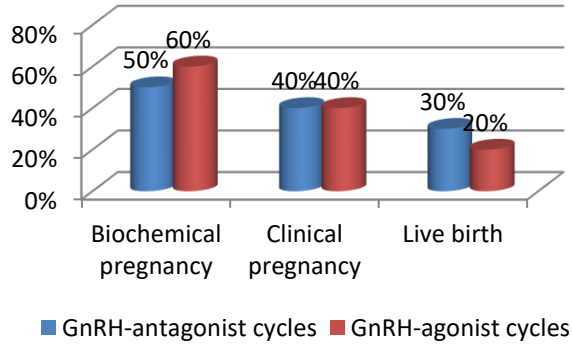

Figure 4 - The outcomes in the L group in the GnRH-antagonist/agonist cycles

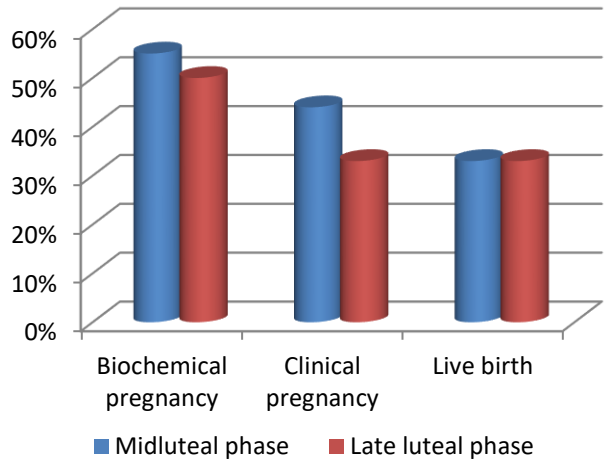

Figure 5. The outcomes in the L group performing the endometrial biopsy in the midluteal/late luteal phase.

Also, the difference between performing the local endometrial injury in the midluteal phase versus the late luteal phase had no statistical signifigance.

We didn't have ovarian hyperstimulation syndrome (OHSS) cases in our study (the most likely explanation is that we had a majority of GnRH-antagonist cycles).

60% of our patients were smokers, but we didn't find any statistical significant difference between the outcomes regarding this criteria.

2 of the 8 patients who obtained pregnancies developed gestational hypertension or preeclampsia, which is more than the 8% in the general population. Therefore we might conclude that the poor endometrial receptivity could be associated with abnormal trophoblast invasion. More studies are needed to confirm or infirm this hypothesis.

At the time of the embryotransfer, the mean endometrial thickness was of 9.4 mm in the C group and of 10.5 mm in the L group This ultrasound feature of the endometrial layer supported the hypothesis of improving the quality of the endometrium by performing the scratching procedure.

As for the secondary outcomes, there were 2 miscarriages in each of the groups but reporting to the number of pregnancies obtained, the miscarriage rate was higher in the C group (for IVF pregnancies, the rate of miscarriage is 20-25%; in the C group we had 40% rate of miscarriage, compared to 28% rate of miscarriage in the L group).

There were 2 multiple pregnancies in the L group and none in the C group.

The incidence of placenta accrete syndrome was cited as less than 0,2% in the general population of pregnant women. In the 8 pregnancies we obtained, we had 1 case of placenta accrete, which proves a rate much higher (again we might conclude that poor endometrial receptivity could be associated with abnormal trophoblast invasion).

Only 4 patients accused spotting after Pipelle biopsy. All patients reported grading less than 4 on the visual analogue scale, therefore we concluded the procedure is painless and does not require pain killers.

Conclusions

One of the main goals in treatment of the couples with recurrent implantation failure is to improve the receptivity of the endometrium. Similar to the previous papers, our study showed that local endometrial injury is of important benefit in women with RIF, when performed in the luteal phase of the cycle prior to IVF.

We also demonstrated that this invasive procedure was most efficient in patients that were not obese and did not have autoimmune diseases or an abnormal uterus.

Another conclusion of our study was that endometrial scratching seemed to be less successful when adenomyosis was present.

Our study also concluded that the rate of gestational hypertension and preeclampsia was higher in women with RIF compared to the general population.

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