GnRH agonist and letrozole in women with recurrent implantation failure

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Reporting of background

The manuscript addresses a very intriguing question of cause of recurrent implantation failure (RIF). RIF for the purpose of research is adequately defined and stated in the manuscript. However, the definition of RIF in current literature is unfortunately not yet standardised (1). The definition states that absence of "implantation" after two or three consecutive assisted reproductive technology (ART) cycles (both fresh and frozen embryo cycles) with total transfer of ≥4 cleavage stage embryos or >2–3 blastocysts of "optimum quality".

The process of "implantation" and embryo endometrial interaction can be explained as apposition, attachment (adhesion) and penetration (invasion). The in vitro fertilisation (IVF) failure could be the result of unexplained subfertility caused by oocyte sperm interaction defects or implantation failures. The oocyte sperm interaction defects are not intended to be considered in implantation failures in ART (as the embryo is optimum quality) so the definition of RIF in ART stands for 4^{+} weeks when the patient is beta human chorionic gonadotropin (HCG) negative that means that there has been no implantation or a 6^{+} weeks beta HCG positive patient in which no intrauterine gestational sac is seen (biochemical pregnancy). The phrase "optimum quality" is also debatable because the quality can be assessed by morphology or by preimplantation genetic testing with next generation sequencing (NGS) based tests.

There is a recent term called as "implantation efficiency" that is a sum of embryo quality, endometrial receptivity and transfer efficiency (EQ + ER + TE) (2). The given constants in the study are EQ and good embryo transfer (TE) techniques so the likely cause for improvement in one intervention arm of letrozole plus gonadotropin-releasing hormone (GnRH) agonist is improvement in ER. Endocrine receptivity can be influenced by anatomical factors, endocrine factors, vascular factors, thrombophilias, vaginal-uterine microbiome derangements and immunological factors (3). The anatomical factors are the endometrial polyps, submucous myomas, uterine septum, synechiae, thin endometrium, adenomyosis, endometriosis, chronic endometritis and hydrosalpinges. These are the exclusion criteria of the present study. The patient had a normal coagulation profile, however the detailed investigations of acquired and hereditary thrombophilias were missing in the study group.

The few other works up is testing for antithyroid antibodies in RIF (4). The study population could also include patients with maternal biochemical studies positive for phospholipid dependent coagulation tests causing RIF. Tests for these include enzyme-linked immunosorbent assay (ELISA) testing for anticardiolipin antibody IgG and IgM and antibody to beta-2 glycoprotein 1 IgG. The coagulation test for lupus coagulant as a cause for RIF can also be done. Furthermore, since the women had RIF they could also have been tested for seronegative

antiphospholipid antibodies (APLA) syndrome. These include testing for antiphosphatidyl serine, prothrombin complex and anti-beta-2 GPI domain I (DI) IgG. Recently it was concluded that women with infertility may represent a subpopulation of patients with undiagnosed systemic autoimmune syndromes in which the main clinical symptomatology is obstetric. Therefore, it was recommended to evaluate APLA in all patients undergoing IVF with the goal to recognise women at high risk of miscarriage (5). Furthermore, there are some causes of inherited thrombophilias like protein S and/or protein C deficiency. There could also be an undiagnosed activated protein C resistance. Other causes of RIF include factor V Leiden gene mutation and factor XIII gene mutation, antithrombin III deficiency, serpine gene polymorphism and prothrombin gene mutations.

In recent reviews there is testing for uterine Natural killer cells in endometrial tissue using immunohistochemistry or flow cytometry (6). Endometrial receptivity array (ERA) determines the window of implantation based on endometrial gene transcription profile (transcriptomic profiling of 238 genes for each patient); "personalised window of implantation". There are primarily two kinds of defects in this window of implantation, the displaced window of implantation and the disrupted window of implantation (7). In displaced window, the RIF is due to asynchronisation of euploid blastocyst apposition and pinopodes expression with no underlying endometrial dysfunction. In pathological disruption of window of endometrial implantation there an aberrant integrin expression (8).

The embryo factor has been excluded as a cause of RIF. The hypothesis is well stated. The description of expected study outcome is given elaborately.

The study population is well defined. The authors have included women younger than 40 years of age fulfilling the criteria of infertility, who had 3 ETs where the initial two "optimum" quality blastocyst ETs could not achieve pregnancy. The study group had documented normal uterine cavity after hysteroscopy. Women with ultrasound detected endometrioma or laparoscopically diagnosed endometriosis were excluded from the study. Women with ultrasound features consistent with adenomyosis, uterine polyps, fibroids, or existing hydrosalpinges were also excluded from the study. The patients with severe male partner infertility was also excluded. All the study participants also had normal thyroid hormone and prolactin profile. The coagulation studies were also normal in the study group.

Reporting of the search strategy

There is also a study published from the same institute with same intervention in the June 2011 to January 2016 period that analysis the participants mentioned in the exclusion criteria (patients with ultrasound diagnosed ovarian endometriomas) (9). This is creditable as the patients in both the inclusion and the exclusion group were studied, analyzed and reported.

Normal endometrium has no aromatase expression (10). Local estrogen synthesis does not take place in eutopic endometrium. This is made possible by proteins that bind with the promoter region of the P450 aromatase gene DNA and inhibit its expression. These inhibitory factors can directly combine with DNA in the promoter regions (inhibitors 1 and 2) or can combine with transcription factors and suppress their action through protein-protein interactions (corepressors 1 and 2). However, in eutopic and ectopic endometrium of patients with endometriosis, these inhibitors and corepressors are decreased or absent. Not only these inhibitors and corepressors are absent, but aberrant proteins replace them (11). These proteins combine with P450 aromatase promoter and the transcription of aromatase gene is active. These proteins can either directly combine with DNA as classical transcription factors (stimulators 1 and 2) or interact with DNA-binding proteins and reinforce their transcriptional activity (coactivators 1 and 2) (12).

Endometriosis has low oocyte quality and implantation rates as a result of aberrant aromatase activity that disrupts the implantation window and alters the balance between progesterone and estrogen in endometrium (13). Changes of eutopic endometrium in endometriosis include changes in glandular and stromal structure, proliferation, alteration in apoptosis and immunity. There is also associated aberrant expression of cell adhesion molecules, proteases, steroids and cytokines and proteins (14). The changes in oocyte cumulus complex and eutopic endometrium of endometriosis patients are summarized in *Table 1*.

The intervention in three arms of study

The three groups of GnRH agonist, GnRH agonist + letrozole and no pretreatment group are well described and the intervention is clearly planned and outlined.

In the second arm of the study, there is addition of

Table 1 Oocyte and endometrial factors in endometriosis with RIF

Cumulus oophorus complex
Oxidative stress markers are significantly elevated in the granulosa cells of oocyte cumulus complex in patients with endometriosis
Oocytes from donors with endometriosis are associated with lower implantation but not clinical pregnancy rates compared to oocytes from normal donors
A decreased number of granulosa cells in the G2/M phase and an increase in both the S phase and apoptotic cells
Eutopic endometrium of endometriosis
Integrin αvβ3 is down-regulated in eutopic endometrium of patients with endometriosis. A key regulator of the αvβ3 integrin is the transcription factor, HOXA10, which is also reduced in mild endometriosis
TNF-α suppresses proliferation of endometrium in healthy women but enhances proliferation of endometrium in women with endometriosis
There is increased heterogeneity in surface epithelium, reduced glandular and stromal mitoses, basal vacuolated cells, reduced thickness, altered neuroendocrine cells. Nerve fibers have also been identified in endometriotic implants

RIF, recurrent implantation failure; G2, gap 2; M phase, mitosis phase; S phase, synthetic phase; HOXA10, homeobox protein A10; TNF-α, tumor necrosis factor alpha.

GnRH analogues. Synthetic GnRH agonists are analogues obtained by incorporating hydrophobic amino acids at specific cleavage points in native GnRH (15). This increases the half-life by increasing the drug resistance to peptidases and increases the affinity to GnRH receptors. The rationale of treatment in this group is to suppress the gonadal function. A prolonged use GnRH agonist protocol down regulates the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion in pituitary and thereby suppresses the endocrine gonadotropin stimulation of ovary and devoid of estradiol (E_2) support the ectopic endometrial implants undergo apoptosis. However, there are several other mechanisms of action of GnRH agonists in endometriosis. There are anti-proliferative and anti-inflammatory effects on endometrial stromal cells. GnRH analogues directly inhibit the endometrial cells by decreasing cell proliferation and enhancing the apoptotic index (16). This is evidenced by studies concluding increased expression of apoptotic proteins Bax and FasL. There is also a decrease in expression of antiapoptotic protein Bcl-2 (17,18). Studies have also shown decrease in the release of pro-mitogenic cytokines like Interleukin-1 alpha and vascular endothelial growth factor (VEGF) (19).

Adding letrozole to GnRH agonist in the third arm of the study prevents the growth of endometriosis in three ways. One is that it suppresses the initial flare rise of E_2 after flare response of increased gonadotropin secretion immediately after GnRH injection (20). Second is that GnRH agonist down regulates the ovarian estrogen production while the letrozole inhibits the conversion of extra ovarian androstenedione (the adipose tissue and skin) to E_2 in endometriosis implants (21). Thirdly, letrozole also blocks all the E_2 synthesis in the endometriosis tissue (21).

E_2 is also produced locally in the endometriosis implant. Androstenedione (A) of adrenal origin is converted to estrone (E_1), which is in turn reduced to E_2 in the peripheral tissues and endometriosis implants. A high level of 17β-hydroxysteroid-dehydrogenase type 1 (reductase) expression in endometriosis implants catalyzes the conversion of E_1 to E_2. E_2 can also induces cyclooxygenase-2 (COX-2) directly and indirectly (through cytokines) resulting in increased concentrations of prostaglandin E_2 (PGE_2) in endometriosis (21). PGE_2 is the most potent known stimulator of aromatase in endometriosis stromal cells. Thus, a positive feedback cycle of continuous E_2 synthesis is established in endometriosis (*Table 2*). The alternative explanations for the observed results are well described by authors.

In 1996, (exemestane, letrozole, and anastrozole), the third generation of aromatase inhibitors achieved approval by the US Food and Drug Administration and has revolutionized the treatment of endometriosis (21,22). The conclusions presented by the study are appropriate for the data presented and within the domain of current literature on management of RIF in IVF.

Conclusions

GnRH analogues have also been shown to alter integrin

Table 2 Pharmacological action of aromatase inhibitors in endometriosis

E_2 is produced from cholesterol through six serial enzymatic conversions in two cell types that cooperate in a paracrine fashion. The rate-limiting two steps
Entry of cholesterol into the mitochondrion, facilitated by the StAR in theca cells
Conversion of androstenedione to E_1 by aromatase in granulosa cells
The aromatase enzyme is located in the endoplasmic reticulum of estrogen-producing cells. There is a single gene for aromatase, which encodes a single protein. Thus, targeting the aromatase protein by specific inhibitors effectively eliminates estrogen synthesis
Endometriosis tissue also has promoter II, which is the proximal promoter responsive to PGE_2 and cAMP, for aromatase expression
There is presence of significant levels of StAR and aromatase activity in ectopic and eutopic endometrium of the patients with endometriosis. PGE2 is the most potent inducer of StAR and aromatase in endometriosis stromal cells
A transcription factor, steroidogenic factor 1, also is aberrantly expressed and binds to steroidogenic promoters in endometriosis tissues. Steroidogenic factor 1 mediates PGE_2-cAMP dependent co-activation of multiple steroidogenic genes, most notably StAR and aromatase genes
The product of aromatase, that is, E_2, is a potent stimulator of COX-2 in uterine endometrial cells. Thus, a positive feed-forward cycle involving StAR and aromatase, E_2, COX-2, and PGE_2 favors continuous formation of E_1 and E_2 and PG in endometriosis
Expression of StAR, aromatase, and other steroidogenic genes enables endometriosis tissue to synthesize E_2 *de novo* from cholesterol
Type III aromatase inhibitors reversibly bind to the active enzyme site, and no enzyme activity is triggered in the endometriosis implants and eutopic endometrium thus breaking the loop

E_1, estrone; E_2, estradiol; PGE_2, prostaglandin E_2; StAR, steroidogenic acute regulatory protein; cAMP, cyclic adenosine monophosphate; COX-2, cyclooxygenase-2.

expression in endometrium (23). Integrins are basement membrane proteins and transmembrane receptors for extracellular matrix (ECM). They are made up of two noncovalently associated subunits, α and β. Increased expression of integrin heterodimer αVβ3 and αVβ5 by letrozole in eutopic endometrium of endometriosis can help in repairing the ER (24). There are studies establishing correlation between integrin expression, endometrial thickness and the ER. The αVβ3 integrin expression is significantly low in disrupted ER. This suggests that under expression of αVβ3 integrin in human endometrium can be the cause of disrupted window of implantation. Letrozole is likely to benefit the disrupted window of implantation in endometriosis. To conclude, this research has avidly proved that aromatase inhibitors in women with endometriosis likely alter the intracellular harmony between estrogen and progesterone action in endometrium, and therefore restores the effect of progesterone in down-regulating estrogen receptors (a critical phenomenon during implantation) (25). As αvβ3 integrin is indirectly regulated by progesterone, this is the most likely cause. Further prospective studies are required to establish these results and to rationalize the use of GnRH agonist and letrozole combination in improving ER in the RIF.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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