Chapter

Endometrial Receptivity in Patients with Polycystic Ovary Syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is a frequent disorder affecting women of reproductive age characterized by infertility. Affected endometrial receptivity seems to contribute to decreased fertility of these patients as suggested by several studies. Understanding the mechanism behind this reduced endometrial receptivity could contribute to discovery of new therapeutic targets for infertility of PCOS. The aim of the paper is to review the current data regarding endometrial receptivity in PCOS patients, the potential mechanisms involved with particular focus on recent findings as the impact of gut microbiota on endometrium, the relationship between vitamin D and endometrial receptivity and the different impact of letrozole and clomiphene citrate on endometrial receptivity in infertile PCOS women.

Keywords: polycystic ovary syndrome, endometrial receptivity, endometrium, implantation, pregnancy

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, having a prevalence of 8 to 13% and of 21% in high-risk groups [1]. Moreover, it is a leading cause of female infertility [2, 3] and the most common cause of anovulatory infertility [4]. A systematic review from 2020 [5] found that there is significant variation in prevalence probably according to ethnic background and design of the published studies, but also to diagnosis criteria used to identify the disease. Thus, they found that the reported prevalence of PCOS vary between 2,2% and 15–20%, with the studies using the Rotterdam criteria reporting the highest prevalence [5].

PCOS has significant consequences on the women health, being associated with infertility, menstrual irregularities, metabolic abnormalities, cardiovascular risk and psychological disturbances [6] and, therefore, impairing the quality of life. The latest guidelines [7] recommend the use of Rotterdam Consensus criteria for PCOS diagnosis, which assumes that two out of the following three features are present: oligo- or anovulation, hyperandrogenism (clinical or biochemical) and polycystic ovaries [8]. The use of these criteria generates several clinical phenotypes with variate impact on reproductive potential and metabolic profile, with some of them diagnosed with difficulty due to a scarce clinical picture. Therefore, PCOS can be a challenging disorder in the reproductive medicine practice.

Hyperandrogenism is a key feature of PCOS, being the result of increased production of both ovarian and adrenal androgens. Ovarian over-secretion of androgens is the consequence of LH stimulation and also the action of high insulin levels on insulin receptors from the ovarian theca cells. Moreover, bioavailability of androgens is increased due to insulin effect to reduce the hepatic production of sex-hormone binding globulin.

2. Fertility in patients with polycystic ovary syndrome

PCOS is considered the most common cause of anovulation, being responsible for 70% of cases of anovulatory infertility [9]. Infertility is a significant complaint among women with PCOS, being reported in 72% of women with PCOS compared with 16% in women without PCOS. However, it seems that the number of children of women with PCOS is similar with those without, suggesting that treatment for infertility is effective [10].

It was also showed that 44% of women with unexplained infertility are probable PCOS cases, underlying that the subtle clinical phenotypes in some of the patients can be the cause of misdiagnosis and, therefore, inappropriate diagnosis and treatment [11].

It seems that the criteria used for diagnosis can also impact the prevalence of infertility which is higher in women with polycystic ovary morphology in patients diagnosed according to Androgen Excess Society criteria (21,7%), while in patients diagnosed according to Rotterdam criteria infertility is present in only 6% of them [5].

Older studies report that 78% of infertile women with PCOS respond to clomiphene citrate (CC) administration [12], with only the remaining 22% requiring alternative therapies, suggesting that anovulation is not the only cause of infertility in PCOS patients. Indeed, even after restoration of ovulation, PCOS patients still have reduced cumulative pregnancy rate and higher rates of implantation failure [13]. Even in cycles with excellent embryos selected for transfer, the success of in vitro fertilization in PCOS patients remain low [14]. In animal models the transfer of blastocysts from normal mice to DHEA-induced PCOS mice resulted in a reduced implantation rate [15]. Moreover, patients with PCOS had an increased risk of miscarriage with reported rates between 30 and 50% of all conceptions [16, 17]. In addition, PCOS seems to be responsible for more than 30% of cases of recurrent miscarriages [18].

Since oocytes and embryo quality do not seems to be the cause of low implantation and pregnancy rate in PCOS patients as demonstrated by donor oocyte models [19, 20], the decreased receptivity of the endometrium seems more probable. Indeed, accumulating data support the hypothesis that endometrium of PCOS patients is affected probably as a consequence of hormonal imbalance. Thus, unopposed estrogens, hyperinsulinemia, hyperandrogenism and the members of insulin-like growth factor family were reported as possible contributors to endometrial pathology in PCOS [21]. Factors associated with unexplained recurrent pregnancy loss like high serum levels of free testosterone and LH, decreased luteal phase progesterone and delayed endometrial development [22] are also found in PCOS patients, suggesting their involvement in the high miscarriage rate in PCOS.

Several abnormalities of the endometrium were reported in PCOS women. Thus, endometrial gene expression and sex hormone receptors, co-receptors, adhesion molecules expression and endometrial markers were reported to be abnormal [23].

3. Endometrial receptivity in women with polycystic ovary syndrome

Endometrial receptivity is a complex feature of the endometrium that allow the embryo to attach and invade the endometrium, and its further development into a viable fetus. For normal implantation both embryo and endometrial quality are important. In normal women the endometrium is receptive to embryo implantation for a period of 3–6 days which starts seven to ten days after ovulation known as the window of implantation. In pathological conditions this window can be shorten or shifted, resulting in infertility or pregnancy loss.

Human endometrium is a tissue whose development depends on the level of circulating hormones. During the follicular phase of the cycle, the increasing circulating estradiol levels determine the proliferation of the endometrial cells and increased endometrial sensitivity to estrogen through increasing the estrogen receptors (ER) ER-α levels [24, 25]. The ER expression is highest in the late proliferative phase, decreasing in the luteal phase [25]. Following ovulation, the progesterone production determines inhibition of cellular proliferation, mitotic activity, DNA synthesis and stimulates the differentiation of the endometrial cells [26]. The inhibition of proliferation of the epithelial endometrial cells is the consequence of the progesterone-induced regulation of genes resulting in down regulation of estrogen receptors and the induction of the enzymes that metabolize estrogens reducing its cellular effects [26]. Moreover, progesterone reduces the expression of androgen receptor in endometrial cells and stroma [26]. As a consequence of all these changes the 'window of implantation' occur. Progesterone is also essential for decidualization, a process that allow trophoblast invasion in case the implantation occur and establish a cytokine milieu and immunomodulatory network in the stroma. The decidualization is the consequence of endometrial stromal cells modifications of the cytoskeleton and up-regulation of prolactin, insulin-like growth factors, IGF binding proteins, insulin receptor and other factors. In the case the implantation does not occur, the decreasing estrogen and progesterone levels determines a shift from the expression of the innate immune genes to inflammatory genes expression in association with cellular apoptosis, increased production of metalloproteinases and prostaglandins, followed by endometrial desquamation and menstruation [26].

In women with PCOS, in the absence of ovulation the progesterone effects on the endometrium are lacking or severely decreased, affecting the decidualization and the window of implantation. Moreover, women with PCOS may have increased exposure to estrogen levels [27] as a consequence of aromatization of increased androgens in adipose tissue and decreased sex hormone binding globulin due to hyperinsulinemia [28, 29]. Several experimental studies support the alteration of endometrium especially in the window of implantation. Thus, Avellaira et al. [30] found that the tissue homeostasis in secretory endometrium of untreated women with PCOS is affected by an imbalance between apoptosis and cell proliferation which is increased as demonstrated by a study evaluating the expression of the proteins related with the two processes [30].

It was also suggested that some endometrial alterations in PCOS are the consequence of prenatal intrauterine exposure to androgens being considered primary endometrial abnormalities [23]. Thus, the endometrium of PCOS women has a preponderance of estrogen and androgen action and decreased progesterone action as a consequence of hormone receptors expression and function.

3.1 Estrogen receptors (ER) expression and function

In PCOS patients the level of ER seems to be increased in all the cycle phases [31–35]. Moreover, some coactivators of the ER-α like TIF2 and AIB1 were also

found in higher levels in the proliferative endometrium of the PCOS patients compared with controls [32–35]. Moreover, during the proliferative phase, the endometrium of PCOS women showed a higher Bcl-2/Bax ratio, indicating the predominance of anti-apoptotic factors in the estrogen receptor increased environment [31]. The higher levels of mRNA and protein for ER-α and coactivators compared with normal women were reported in the mid-secretory phase endometrium of PCOS as well [32]. Moreover, the coactivator ARA70 was increase and epithelial expression of beta3-integrin, a protein involved in cell adhesion and cell surphace mediated signaling, was decreased in endometrium of PCOS versus control [32].

Enhanced ER-α activation was associated with rare or absent apoptosis and increased in cell proliferation in the endometrium in the mid luteal phase [30, 36–38]. On the other hand, it is possible that high circulating estrogens as in patients performing ovarian stimulation to determine the apoptosis of the endometrial glandular cells, as showed by an experimental study by Chen et al. [39].

Other factors can contribute to increased exposure of endometrial cells to estrogens. Thus, the modified activity of the endometrial enzymes 17-β-hydroxysteroid dehydrogenase, which is downregulated and hydroxysteroid dehydrogenase type 1, which is upregulated, could contribute to increased local production of estradiol and androstenediol with increased estrogenic activity [40–42].

3.2 Progesterone receptor (PR) expression and function

In endometrium of PCOS women progesterone receptors (PR) alpha and beta mRNA is overexpressed and the stromal immunostaining of PR-b and Ki67 is higher compared with BMI-matched controls [43]. Moreover, an imbalance between PR-a expression and PR-b was found in proliferative endometrium of obese PCOS women, with the predominance of the later [43]. It was also reported that endometrial tissue of PCOS patients has decreased responsiveness to progesterone [44], also known as 'P resistance' [45]. This progesterone resistance might be due to reduced binding and activation of PR [46] or to an altered expression of its isoforms [46, 47]. Elevated PR isoform expression was showed to be associated with increased systemic levels of estrogen [48] and of androgen [49, 50]. Thus, hyperandrogenism modulates the expression and function of PR being associated with inactive or less active isoforms of PR [49–51], being probably responsible for progesterone resistance in PCOS women.

Mucin 1 (MUC1) is a progesterone-regulated molecule that carries selectin ligands recognized by the human blastocyst. Thus, Margarit et al. [52] showed that MUC1 expression is lower in anovulatory PCOS than in fertile patients, being a possible contributor to decreased implantation.

3.3 Androgen receptor

In the endometrium of PCOS women the androgen receptor (AR) mRNA and protein expression is increased [23] and coactivators of AR were found to be overexpressed as well [37]. Among these coactivators, Melanoma-associated antigen 11 (MAGEA11) was showed to bind to AR resulting in alteration of window of implantation [11]. Endometrial microenvironment can also contribute to over-exposure to androgen. Thus, low level of SHBG can increase the bioavailable testosteron at endometrial level [53] and increased activity of endometrial 5a-reductase generates potent androgens such as di-hydro-testosterone [37]. The uptake of intracrine precursors of testosterone [38, 54] and increased activity of hydroxi-steroid-dehydrogenase might contribute to high androgen exposure of endometrium in PCOS [55].

3.4 Hyperandrogenism

During the mid-secretory phase of the menstrual cycle and the window of implantation the level of circulating androgens reach a nadir in normal cycles [56], while in PCOS patients with hyperandrogenism the level of circulating androgens is constantly increased. There is a significant body of evidence suggesting that overexposure to androgens may affect the normal development of endometrium and, therefore the endometrial receptivity. Thus, Cermik et al. [57] studied the effect of testosterone on HOXA10 expression in endometrium, a gene well known to be involved in endometrial receptivity. They demonstrated that, in vitro testosterone exposure decreases the expression on HOXA10 and prevents the increase of this gene in response to estrogen and progesterone [57]. They also confirmed that the expression of HOXA10 is decreased in the endometrium of the hyperandrogenic PCOS patients [57]. Homebox (HOX) are genes essential for endometrial receptivity which are maximally expressed in endometrium during the window of implantation [58]. Their importance in implantation is demonstrated by experimental studies on female mice with disruption of HOXA10 which show infertility with implantation failure in spite of the presence of ovulation [59].

It was also showed that Wilms tumor suppressor (WT1) gene which is expressed in endometrium in the window of implantation, was downregulated in ovulatory women with PCOS in comparison with normal controls and that this downregulation is the consequence of androgen exposure in in vitro models [60] Since high androgen are associated with elevated PR isoforms, it was suggested that hyperandogenism could be a contributor to progesterone resistance found in PCOS women [50, 61]. High androgens can also affect the number and function of endometrial pinopodes which are associated with endometrial receptivity [62, 63]. Androgens can also influence the decidualization by their ability to modulate the oxidative stress response in decidualized endometrial cells [64] since the oxidative stress was showed to influence factors involved in embryo implantation like cytokeratin 8 (CK-8) [65]. Other androgens can also act on endometrium. Thus, DHEA seems to block glucose utilization resulting in inhibition of decidualization [66] and modulation of cell survival and apoptosis [67]. In pregnant mice treated with DHEA an impaired LIF-signal transducer and activator of transcription 3 (STAT3) pathway was observed, which was associated with implantation failure [68].

Clinical data showed that pregnant women with PCOS have lower endovascular trophoblast invasion in relation to circulating testosterone and the clinical phenotypes involving hyperandrogenism [69, 70].

3.5 Hyperinsulinemia and insulin resistance

In clinical studies, in PCOS patients undergoing in vitro maturation-in vitro fertilization embryo transfer cycle insulin resistance was associated with decreased implantation, clinical pregnancy and ongoing pregnancy rates [71]. Energy metabolism is vital for proper endometrial function taking into consideration the rapid turnover of endometrial tissue. Therefore, insulin action on endometrial tissue might be essential for endometrial receptivity. Insulin resistance and compensatory hyperinsulinemia can have detrimental effects on the implantation process as hyperinsulinemia has been shown to impair stromal cell decidualization in vitro [72–74]. In PCOS endometrium several abnormalities in insulin signaling and glucose transport have been reported. Thus, hyperinsulinemia can reduce insulin receptor substrate 1 (InRS-1) activation and glucose transport in endometrial stromal cells in PCOS women [75–76], probably via inflammatory pathways [76, 77]. Experimental studies showed that in vitro exposure to dihydrotestosterone altered the expression

of insulin receptor and insulin receptor substrates and the phosphorylation of insulin receptor in endometrial stromal cells [74]. Other studies suggests that insulin action is decreased in endometrium of hyperinsulinemic women with PCOS, by showing that pAS160T642 and SLC2A4 which are substrates of insulin receptor are decreased in comparison with non-hyperinsulinemic PCOS and controls [75].

In vitro studies showed that insulin inhibits the production of IGFBP-1 in the endometrial stoma. IGFBP-1 is considered a biomarker of decidualization [73]. It is also possible that adiponectin, an insulin regulating molecule and regulator of glucose metabolism, which is decreased in PCOS patients, to be involved in endometrial receptivity of these patients. Thus, adiponectin receptors were found to be highly expressed in the human endometrium during the window of implantation [78]. Moreover, decidualized mouse endometrium is able to secrete adiponectin and adiponectin receptors were found both in decidual cells and embryo after implantation [79]. This data supports the hypothesis that adiponectin might play a role in endometrial receptivity and implantation. Studies showing that metformin reduces the miscarriage rates in PCOS patients indirectly support the hypothesis of insulin resistance involvement in endometrial receptivity [80].

3.6 Vitamin D

Vitamin D deficiency is a frequent condition among women with infertility or PCOS. Numerous studies suggested a role of vitamin D in reproductive health at variate level of reproductive system, including endometrium. However, clinical data regarding the association between serum vitamin D level and endometrial pathology are divergent. A prospective controlled study [81] analyzed factors associated with recurrent implantation failure and found higher prevalence of chronic endometritis, a lower vitamin D level and a borderline lower progesterone level in comparison with controls [81]. However, two recent systematic reviews found no association between serum vitamin D level and miscarriage rate in women who performed in vitro fertilization [82, 83], while one of these reviews reported higher pregnancy rates in vitamin D replete patients undergoing assisted reproduction treatments [82].

Experimental studies support the role of vitamin D in endometrial receptivity. Thus, Guo et al. [84] found that circulating level of vitamin D was positively associated with vitamin D receptor and HOXA10 protein level expression in the endometrium and these were substantially elevated in pregnant women compared to non-pregnant women. During the window of implantation, higher serum vitamin D levels were associated with more mature pinopodes (84). Another study showed that in patients with recurrent implantation failure vitamin D treatment increases progesterone receptor mRNA and protein level and its phosphorylation on Ser294 residues in the endometrial cells (85). These results suggest that vitamin D may play a key role in the endometrial receptivity [85].

In patients with PCOS vitamin D deficiency is frequently found [86]. However, specific data regarding the relationship between serum vitamin D and endometrium in PCOS patients are scarce. Several studies reported that vitamin D treatment can improve the features associated with endometrial receptivity like hyperandrogenism [87], hyperinsulinism and insulin resistance [88], inflammation and oxidative stress [89].

In an experimental study in a rat model of PCOS it was found that immunohistochemical staining of caspase-3 and Ki-67 were decreased with vitamin D treatment compared non-treated group [90]. Moreover, endometrial, epithelial and stromal thickness measurements were decreased in the vitamin D treatment group compared to non-treated PCOS group [90].

4. Strategies to improve endometrial function in PCOS

4.1 Ovulation induction agents

First-line treatment for ovulation induction in the treatment of infertility in PCOS are oral agents, with letrozole being superior to clomiphene citrate (CC) in terms of endometrial thickness and markers of endometrial receptivity [7, 91, 92]. Gonadotropins are the second line treatment and it seems that they have a less deleterious impact on endometrium [93]. However, no data today support the ovulation induction in infertile ovulatory PCOS for the modification of endometrial receptivity.

Several studies evaluated the different impact of ovarian stimulation with letrozole versus clomiphene citrate in patients with PCOS. Thus, Wallace et al. published in 2011 a randomized controlled study which reported that letrozole positively influenced a number of markers of endometrial receptivity like mRNA expression of leukemia inhibitory factor, dickkhopf homolog 1, fibroblast growth factor 22 compared with CC [94]. Another randomized controlled study which included 160 patients diagnosed with PCOS found that indices of endometrial receptivity like the volume, vascularization index, flow index and vascularization flow index of endometrium on the day of hCG administration and 7–9 days after ovulation were significantly increased in letrozole group compared with CC [95]. Moreover, the biochemical pregnancy rate, clinical pregnancy rate and ongoing pregnancy rate in letrozole group were significantly higher compared with CC group [95]. The same authors evaluated the indices of endometrial receptivity in treated PCOS patients (Letrozole or clomiphene citrate) and non-treated [96]. They noticed that, although the successful ovulation rate did not differ between the letrozole group and CC group, endometrial thickness, endometrial volume, vascularization index, flow index, vascularization flow index, integrin αvβ3 and VEGF concentrations in uterine fluid were significantly higher in the window of implantation in the letrozole group compared with the CC group and natural cycle group [96]. Moreover, the clinical pregnancy and ongoing pregnancy rates of the letrozole group were significantly higher than in the CC group [96]. The markers of endometrial receptivity analyzed were significantly higher in pregnant patients [96]. The endometrial flow index during the implantation window had the highest predictive value for pregnancy. The integrin αvβ3 in uterine fluid had better predictive value than VEGF [96].

In an experimental study on female rats it was found that the expression of integrin αvβ3 in the clomiphene citrate group was lower than in the letrozole and saline solution groups [97]. The expression of HOXA10 was statistically significantly higher in the saline solution group than in the letrozole group, and the letrozole group showed a statistically significantly higher expression of HOXA10 compared with the clomiphene citrate group. The authors concluded that, in rats, letrozole affects the expression of HOXA10 in uterine epithelium but has no effect on the expression of integrin αvβ3, which suggests that clomiphene suppresses endometrial receptivity more than letrozole.

4.2 Antiandrogens

Antiandrogens as flutamide, finasteride and spironolactone are used in clinical practice to improve the signs of clinical hyperandrogenism like hirsutism, acne, alopecia. Animal studies demonstrated the ability of antiandrogens to improve endometrial function [98, 99]. However, their use in patients seeking infertility treatment is prohibited by their teratogenic effects, being generally recommended to be administrated only in association with oral contraception [100]. Another way

to improve hyperandrogenism is by administration of combined oral contraceptives which decrease ovarian androgen production and decrease androgen bioavailability through stimulation of sex hormone binding globulin production. Indeed, oral contraceptives, administrated in a successive or unsuccessive manner before in vitro fertilization, were showed to increase the implantation and pregnancy rate and reduce the risk of pregnancy complications along with significant decrease in circulating androgens [101]. However, in a randomized controlled trial ovulation rate and live birth rate were superior in PCOS patients receiving life style modification with or without oral contraceptive in comparison with oral contraceptive alone [102].

4.3 Freeze all strategy

Elective frozen embryo transfer after a freeze-all strategy was suggested for infertile PCOS patients undergoing IVF [103]. This approach could be particularly effective in PCOS due to exposure of the endometrium to high estrogen levels during controlled ovarian stimulation. Thus, transfer of the embryo in a subsequent cycle may avoid failure of implantation due to inadequate endometrium receptivity. This strategy was showed of particular benefit in PCOS patients and only in case of more than 16 oocytes or estradiol higher than 3000 pg./ml, resulting in higher live birth rate [104]. Regarding the preparation of endometrium, it seems that the pregnancy rate is higher in natural cycles over the hormone replacement cycles in women performing frozen embryo transfer [105]. However, specific data for PCOS women are lacking.

In women with PCOS, no difference in live birth/ongoing pregnancy and clinical pregnancy rates was detected between FET cycles stimulated with hMG and cycles artificially prepared with E2 valerate [106–108].

4.4 Gut microbiota and endometrial receptivity

There is accumulating evidence that gut microbiota might play a role in PCOS pathogenesis [109, 110]. Thus, it was showed that gut microbiota of PCOS patients is different from controls [111] and that variate profiles of gut microbiota are associated with features of PCOS such as hyperandrogenism [112], hyperinsulinemia and insulin resistance [113] and obesity [114]. In experimental studies transplantation of feces from PCOS women leads to development of PCOS features in mice [109] and oral administration of variate bacteria was associated with improvement of PCOS manifestation [110]. Thus, Qi X et al. [109] showed that Bacteroides vulgatus was markedly elevated in the gut microbiota of individuals with PCOS and that transplantation of fecal microbiota from women with PCOS or B. vulgatus-colonized recipient mice resulted in increased disruption of ovarian functions, insulin resistance, altered bile acid metabolism, reduced interleukin-22 secretion and infertility [109]. It was also showed that IL-22 improved the PCOS phenotype [109]. Guo Y et al. [110] showed that PCOS rats displayed different composition of gut microbiota that in the controls. Lactobacillus, Ruminococcus and Clostridium were lower while Prevotella was higher in PCOS rats when compared with control rats. After treating PCOS rats with Lactobacillus and fecal microbiota transplantation from healthy rats estrous cycles were improved with decreasing androgen biosynthesis [110].

However, it is possible that the relationship between gut microbiota and PCOS to be bidirectional since in androgen-induced PCOS animal models the disturbances of gut microbiota was reported [115, 116]. In turn, DHEA-shaped gut microbiota transplanted to pseudo germ-free rats recipients trigged disturbances in

reproductive hormone [115]. Another study showed that both androgens and high fat diet could shift the overall gut microbial composition, being associated with the development and pathology of PCOS by shaping gut microbial communities [117]. Although direct evidence of a connection between gut microbiota and endometrial receptivity was not reported yet, we can hypothesize a connection between these two due to the recognized involvement of insulin resistance, hyperandrogenemia and obesity in endometrial receptivity which, in turn, can be shaped by intestinal bacterial community.

Regarding the mechanisms connecting gut microbiota and PCOS, Tremellen and Pearce suggest that dysbiosis of the gut microbiota brought about by a high fat-sugar diet in PCOS patients leads to an increase in intestinal permeability. Lipopolysaccharide produced by Gram-negative bacteria traverse the gut wall to enter the circulation, leading to a chronic state of low-grade inflammation. Activation of the immune system interferes with insulin receptor, driving up insulin levels, which boost testosterone production in the ovary, leading to PCOS [118].

Another way by which gut microbiota might influence the endometrial receptivity is through its recently demonstrated close connection with genital tract microbiota [119–122]. The crosstalk between gut and vaginal microbiota is highlighted by studies showing that oral administration of probiotics or bacteria can influence immunity in the vagina [120]. Thus, oral administration of Lactobacillus johnsonii was showed to inhibit the expression of inflammatory molecules in the vagina and to alleviate Gardnerella vaginalis induced vaginosis [122]. Kutteh et al. [121] demonstrated that intestinal tract immunization by oral and rectal route is followed by the induction of specific antibodies in human female genital tract secretions [Kutteh 2001]. Kim et al. [120] showed that in ovarectomized female mice, the oral administration of anti-inflammatory *Lactobacillus plantarum* NK3 and Bifidobacterium longum NK49 from kimchi and human fecal lactic acid bacteria collection was associated with alleviation of Gardnerella vaginalis induced vaginosis, inhibition of NF-κB activation and TNF-α expression in the vagina and uterus, and decreased the Gardnerella vaginalis population in the vagina [120]. In turn, Gardnerella vaginalis-induced vaginosis increased colonic myeloperoxidase activity, TNF-α expression, and fecal Proteobacteria population. NK3 and/or NK49 treatments reduced TNF-α expression and NF-κB activation in the colon and restored Gardnerella vaginalis disrupted gut microbiota composition [120].

This interplay between gut and reproductive tract microbiota may be related with endometrium function since, recently, it was showed that pathological modification of the profile of the bacterial community of the endometrial fluid might play a role in poor reproductive outcome for in vitro fertilization patients [123]. Thus, the presence of a non-Lactobacillus-dominated microbiota in a receptive endometrium was associated with significant decreases in implantation, pregnancy, ongoing pregnancy, and live birth rates [123]. Haahr et al. [124] found a decreased pregnancy rate in patients with abnormal vaginal microbiota performing IVF [124]. These studies highlight the importance of reproductive tract microbiota in fertility, possibly by modulation of endometrial receptivity.

All these data offer future therapeutic strategies to counteract decreased endometrial receptivity in PCOS women by modulating gut or reproductive tract microbiota or by administration of interleukin 22 as suggested by Qi X et al. [109].

4.5 Assessment of endometrial receptivity and therapeutical implications

The evaluation of endometrial receptivity was historically done by endometrial histology. However, recent studies find little concordance with new transcriptomic methods of endometrial receptivity assessment [125]. Endometrial receptivity array

(ERA) is a test that analyzes the gene expression of the endometrium using a panel of 238 genes that have been implicated in endometrial receptivity. The indication of this test in the clinical practice is to identify the window of implantation in patients with accelerated or delayed endometrial luteal phase development, therefore allowing the transfer of the embryo in the right moment for the embryonic-endometrial synchrony. However, data regarding the benefit of this test are limited [126]. Although in PCOS patients performing IVF ERA test has hypothetical indications, studies evaluating this aspect are lacking.

Another aspect that should be clarified further is the consistency of the ERA findings from cycle to cycle since the transfer of the embryo should be done in a non-biopsy cycle. This aspect was analyzed in seven women who performed the ERA test in separate endometrial samples obtained 29–40 months apart and found high similarity across the samples [127]. However, only five samples were from the luteal phase and only four were in the receptive phase, all the samples being normal. Therefore, the possibility to extrapolate these findings to patients with endometrium receptivity abnormalities or dyssynchrony is unknown.

The ERA test was studied in several categories of infertile patients. Tan et al. [128] found that among patients with a history of at least one euploid blastocyst implantation failure personalized frozen embryo transfer (FET) according to ERA test results was superior to standard FET in terms of implantation and ongoing pregnancy rate, although the difference was not statistically different [128]. Other studies showed a similar pregnancy rate between patients with receptive and non-receptive endometrium according to the ERA test when a personalized embryo transfer was performed in patients with recurrent implantation failure [129] or with a history of implantation failure [125]. On the other hand, Eisman et al. [130] found a similar pregnancy rate in women who had a prior failed embryo transfer and personalized frozen embryo transfer following ERA test compared with women without a prior failed embryo transfer [130]. However, the pregnancy rate was lower in patients with more than three failed embryo transfer despite personalized transfer [130].

A multicenter, open-label randomized controlled trial evaluated patients at their first appointment for IVF after exclusion of patients with recurrent miscarriage and implantation failure. They found that a personalized frozen embryo transfer guided by ERA test was superior in terms of cumulative pregnancy and live birth rate to standard frozen embryo transfers and fresh embryo transfers [131]. At the first embryo transfer cycle, although the pregnancy rate and implantation rates were significantly higher compared with the two groups without ERA guidance, the live birth rates were similar [131]. Basil et al. [132] performed a single center retrospective cohort study and found that using the ERA test in patients undergoing frozen embryo transfer, there is no benefit in terms of ongoing pregnancy rate in good prognosis patients [132].

Taken all together, these studies suggest that the ERA test might be beneficial in patients with implantation failure, although future studies are necessary to clarify this aspect. In patients at their first attempt of IVF personalized frozen embryo transfer guided by ERA test may increase the cumulative pregnancy and live birth rate, although without obvious benefits at the first transfer. Whether these results are also valid for PCOS patients remains to be established.

5. Conclusion

An increasing body of evidence suggests that endometrial receptivity in PCOS patients is decreased, being a significant contributor to infertility in these patients. However, specific strategies to overcome this barrier in infertile PCOS women should be created in order to improve fertility treatment outcome.

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