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Reasons and Mechanisms of Recurrent Failed Implantation in IVF

Violeta Fodina, Alesja Dudorova and Juris Erenpreis

Abstract

Recurrent pregnancy loss (RPL) and recurrent implantation failure (RIF) are serious problems in IVF and ICSI cycles. Different factors are showed to be responsible for these clinical challenges – such as paternal, maternal, embryonic, immunological, infectious, hormonal, and others. In this chapter we have tried to review the available data on reasons for the RIF, and systematize them into: 1) uterine factors; 2) embryo factors; 3) immunological factors; 4) other factors. Interplay between all these factors play a role in RIF, and further investigations are needed to elucidate their significance and interactions – in order to elaborate more definite suggestions or guidelines for the clinicians dealing with artificial reproductive techniques and facing RPL and RIF.

Keywords: IVF, failed implantation, embryo loss, pregnancy loss

1. Introduction

1978 was the year when the first IVF baby Louise Brown was born. From that time reproductive technology progress grew exponentially as well as the experience in current field. In the next years after Louise Brown birth initial implantation rates were < 5% per embryo [1]. As ART technology progressed, many clinics replaced cleavage-stage embryos to blastocyst-stage embryos, and switched from multiple embryo transfers to double- or single-embryo transfers. Each of those achievements led to the point where modern reproductology stands.

Despite the accumulated experience and knowledge, there are many medical questions that need to be answered, because recurrent pregnancy loss (RPL) and recurrent implantation failure (RIF) still exist.

RPL is a disorder defined by the American Society for Reproductive Medicine (ASRM) as the loss of two or more consecutive clinical pregnancies until 20 weeks of gestation [2]. It is known that around 5% of all women are experiencing two consecutive pregnancy losses, 75% of which are implantation failures [3]. In the case of RIF, because of rapidly changing field of ART there has been always a lack of consensus on the definition of RIF, and up till today the definition of RIF is still not unanimous.

One of the first attempts to define RIF was done by Coulam twenty years ago. He defined RIF as a failure to achieve pregnancy with more than 12 embryos transferred in several procedures [4]. During the consecutive 20 years, more and

criteria have been added. A parameter of the blastocyst in the definition of RIF was introduced in 2007: it has been stated that for RIF to be diagnosed, in the patients history there should be the transfer of ≥ 8 of the 8-cell embryos, the transfer or ≥ 5 blastocysts without achieving the pregnancy [5]. After that the researchers started to specify that good-quality embryos is also a significant factor that should be taken into account [6]. Good-quality embryo was defined as having the correct number of cells corresponding to the day of its development and day-5 embryos (blastocysts) were graded according to expansion and quality of the inner cell mass and trophectoderm [7]. Coughlan with colleagues in 2014 proposed definition in which they also added the age of women [8]. About the same time, Lukasz with co-workers stated that RIF should be defined as the absence of implantation defined by a negative serum hCG 14 days after oocyte collection, after two consecutive cycles of IVF, ICSI or frozen embryo transfer, where the cumulative number of transferred embryos was no less than four for cleavage-stage embryos and no less than two for blastocysts, with all embryos being of good quality and of appropriate developmental stage [9]. The PGD Consortium, a specialized group of European Society of Human Reproduction and Embryology, suggested one of the last definitions of RIF: it is a failure to achieve pregnancy after ≥ 3 embryo transfers (ET) of high-quality embryos in women < 40 years, or transfers of ≥ 10 embryos in total in multiple transfers. Presence or absence of pregnancy is diagnosed by an ultrasound examination after the 5th week [8, 10, 11]. Implantation failure can depend on different factors. Successful embryo implantation is an interactive process between the blastocyst and the uterus. Synchronized development of embryos with uterine differentiation to a receptive state is necessary to complete pregnancy. Implantation failure may occur even on early stages during the embryo attachment or migration. As a result, there will be no objective evidence of a pregnancy, i.e. negative urine or blood pregnancy tests (negative hCG) [12]. Another scenario - embryo can migrate through the luminal surface of the endometrium and start to produce hCG, which may be detected in the blood or urine. But even on this stage the process could be disrupted before the formation of an intrauterine gestational sac. In general, implantation failure is usually distinguished into two groups. The first group included women who never shown quantifiable signs of implantation, such as increased levels of hCG. The second group include women who have an evidence of implantation (detectable hCG production) but it did not proceed beyond the formation of a gestational sac visible on ultrasonography 2 weeks later [8]. From the clinical point of view, as defined by the ASRM, implantation is considered successful when there is ultrasonographic evidence of an intrauterine gestational sac or by histopathological examination [2]. With vast numbers of potential causes to consider, to diagnose an etiology of implantation failure is still a complex task for every reproductologist. Some researchers attempted to present summarized reasons of RIF. For example, Timeva et al. have divided RIF causes in three main groups: 1) multifactorial RIF with the subgroups of maternal or paternal factors, hormonal or metabolic disorders, infections and thrombophilias; 2) endometrial RIF that is caused due to thin (≤ 6 mm) endometrium, with or without variations in vascularity; 3) idiopathic RIF, which is unexplained failure to achieve pregnancy after transfer of good quality embryos, without any anatomical and histological changes in uterine cavity and endometrium, without any other disturbances in patient, patient-partner and embryos [13]. Some other authors, in turn, have distinguished etiologic groups such as decreased endometrial receptivity, defective embryonic development and also multifactorial effectors, including into the multifactorial group endometriosis, hydrosalpinges and suboptimal ovarian stimulation [6]. However, there are two main causes of implantation failure that are always present in the majority of all the classifications: uterine and embryo factors. Therefore, we

will shortly review these two, and will also add some data on the immunological and other factors of interest in the context of RIF.

2. Uterine factors

2.1 Endometrial receptivity

Endometrial environment plays a crucial role in embryo implantation and early placental development. There is a certain period of endometrial maturation during which the trophectoderm of the blastocyst can attach to the endometrial epithelial cells and subsequently proceed to invade the endometrial stroma, which is called endometrial receptivity [14]. This complex process provides the embryo with the opportunity to normally attach, implant and develop.

There is a short period of time during the menstrual cycle, when the endometrial receptivity is optimal and embryo implantation is possible. This period is called “window of implantation” (WOI). Studies with donor embryos in humans have shown that this receptive period starts at day 6 post ovulation and continues 4–5 days (or 3–6 days) within the secretory phase (day 20–24 of the menstrual cycle) in most healthy women [15, 16]. Endometrium is unique in its ability to block embryos from implanting, except during this narrow window of receptivity, where endometrium undergoes morphological, cytoskeletal, biochemical, and genetic changes [17]. As shown in the mouse models (and is also true in the other species), WOI is regulated by ovarian steroid hormones. In the receptive endometrium, crucial hormones are progesterone and 17 β -estradiol [18, 19]. In certain pathologic conditions, this window is narrowed or shifted, which disrupts normal implantation, leading to infertility or pregnancy loss [15, 16].

2.2 Human endometrium transcriptomics

The transcriptome reflects the genes that are actively expressed at any given time within a specific cell population or tissue [20]. Human endometrial receptivity transcriptome is a rather complex issue because the quantity of crucial genes that plays a main role in receptivity is still a debatable question. Despite so many publications that revealed hundreds of simultaneously up- and down-regulated genes, the number of selected genes usually differs from one publication to another.

The early search using mouse models started with a few identified genes of receptivity, such as leukemia inhibitory factor-LIF, Homeobox protein X3, genes of embryo response- Cyclooxygenase 2-COX 2; and decidualization -Interleukin 11 Receptor-IL-11R [20]. In 2003 from comparing the gene expression pattern of 375 human cytokines, chemokines, and related factors in receptive and prereceptive human endometrium identified IGF-1 (insulin-like growth factor-binding protein) as a new endometrial receptivity gene [21]. Furthermore, Zhang et al. proposed 148 receptivity biomarkers [22]. Tapia et al. suggested a list of 61 receptivity biomarkers [23]. Bhagwat et al. found 179 genes that have the potential to be called Receptivity Associated Genes [24]. In an enrichment analysis used to identify a meta-signature of highly presumed biomarkers of endometrial receptivity, a statistically significant meta-signature of 52 up-regulated and five down-regulated genes was identified [25]. The highest scores in receptive-phase endometrium reserved 5 up-regulated transcripts - GADD45A, SPP1, PAEP, GPX3 and MAOA. The five down-regulated transcripts receptivity-associated genes were SFRP4, EDN3, OLFM1, CRABP2 and MMP7 [22–24, 26, 27]. Interestingly, commercial Endometrial receptivity array (ERA test) by Igenomix [28, 29] shares 47 genes in common with the identified 57 putative receptivity biomarkers.

As the potential biomarkers for endometrial receptivity, many other molecules have been also studied - like mucin (MUC-1), trophinin, L-selectin, Wingless (Wnt) family members, etc. [30].

2.3 Endometrial receptivity Array

Endometrial receptivity array was developed and patented in 2009. The group of Garrido Gomez from Igenomix have developed a clinical algorithm with a computational predictor which test results are based on the expression analysis of 248 genes [29]. Expression profiling is accomplished by assaying mRNA levels with microarrays or next-generation sequencing technologies (RNA-seq), that allowed identification of the transcriptomic signature of the window of implantation [31]. The idea is to detect a specific point in time of endometrial cycle in which the WOI starts, allowing physicians to perform personalized embryo transfer (pET). The accuracy and consistency of the ERA test had been demonstrated in several trials, that showed that the ERA test is a reliable and reproducible method for determination of the exact time of the WOI that can be used with better results in comparison to histological dating of endometrial receptivity [32]. A pilot study was conducted by Igenomix of 17 RIF patients, who underwent oocyte donation and routine embryo transfer (ET) but were then treated with pET after the personalized diagnosis of their WOI. This study demonstrated that embryo-endometrial synchronization within an optimal time-frame increases the chances of success in an assisted reproductive treatment [33]. The same group showed that patients with at least three previous failed oocyte donation cycles, and IVF patients aged <40 years, with at least three failed IVF cycles with a receptive ERA diagnosis resulted in a 62.8% pregnancy rate [20]. Other groups also showed increased probability of having successful implantation and pregnancy after performed pET in accordance to the ERA result. Results in the Indian population revealed an endometrial factor in 27.5% of the RIF patients, which was significantly greater than 15% in the non-RIF group [17]. Increased percentages of non-receptive ERA test in women with RIF have been also demonstrated [17, 28, 34]. However, the data on the ability of the ERA test to improve the implantation chances in RIF patients are conflicting, with some studies showing no beneficial effect of the test [35, 36]. Also, some studies failed to demonstrated concordance between the ERA test and histological dating of the endometrial biopsies [37].

2.4 Uterine microbiome

Normal microbiome in healthy women primarily consists of hydrogen peroxide-producing Lactobacilli species [38]. During infancy the vaginal flora consists of aerobic and anaerobic bacterial populations, including Streptococcus and Staphylococcus, Prevotella and Enterobacteria species [39]. When puberty comes, the estrogen production causes glycogen to rise and pH to decrease with subsequent domination by Lactobacilli species. Microbiomes of all reproductive organs (vagina, cervix, Fallopian tubes, and ovaries) are significantly correlated [40]. It has been suggested that instead of a single most frequent microbiome, there are multiple core microbiomes: either dominated by variety of Lactobacillus species, or with a lower percentage of Lactobacilli and dominance of anaerobic bacteria [38].

Studies indicate that lower diversity in the microbiome show better outcomes [41–43]. It seems that gravid vaginal microbiome tends to be more stable and less diverse through all the period of pregnancy [44] with major change such as an increase in the dominance of four Lactobacillus spp. (*L. crispatus*, *L. jensenii*, *L. gasseri*, and *L. vaginalis*) and a decrease in the amount of anaerobic species [45].

Regarding the endometrial microbiome of women with RIF, Bacteroides and Proteobacteria seem to be the most represented [46]. Meta-analysis that was done in 2013 also proved that dysbiotic shifts are more frequent in subfertile population [47]. Also, a contamination from the transfer catheter tip by Enterobacteriaceae, Streptococcus, Staphylococcus, *Escherichia coli* and Gram-negative bacteria has a negative effect on implantation and pregnancy rates [48–52].

3. Embryo factors

3.1 Embryo quality

Blastocysts (day-5 embryos) are graded according to expansion and quality of the inner cell mass and trophectoderm. Other criteria include blastomeres of equal size and regular in distribution, distribution of cytoplasm without granularity and less than 10% fragmentation [7]. Good-quality embryo needs to have the correct number of cells corresponding to the day of its development. To make this statement and describe all the pathological elements in development, at least 4 decades of research was needed to achieve the modern stage of embryo evaluation such as time-lapse imaging.

At early stages a morphological evaluation of the embryo was the only criteria for estimating developmental potential of embryo and predicting the probability of achieving pregnancy [53]. The history behind the science of current time-lapse embryo imaging goes back to 1997 when Payne et al. documented the use of time-lapse video cinematography in order to observe the initial events in 38 normally fertilized human oocytes and compare these events with the day 3 embryo development [54]. After ten years based on Payne et al. work, Mio and Maeda extended the analysis period to blastocyst stage and obtained 286 images of human oocytes and embryos [55]. At the same year, Lemmen and colleagues analyzed the events that occur during the first day of the development after fertilization of 102 oocytes by using a microscope with an enclosed camera system [56]. They were the first group that paid attention to embryo kinetic properties, by establishing a link between the early disappearance of pronuclei after fertilization, early first cleavage, and many blastomeres on day 2 of the development. After some period of time many authors started suggesting that kinetic properties need to be added to the morphological evaluation, like timing of embryonic cell divisions [57, 58].

At first the main focus was on time of first embryonic division or early cell division after which embryo becomes 2-cell organism. Correlation between pregnancy rate and time of early cell division was first studied by Edwards group [59]. They concluded that the transfer of embryos that cleaved 24–26 h after fertilization results in higher implantation and pregnancy rates compared to embryos with delayed division. Later many other publications showed that too fast or too slow cleavage has a negative impact on embryo development [60–62].

Modern time-lapse observation systems are developed for more optimized and accurate selection of viable embryos that includes morphological grading with the possibility to register kinetic parameters [60]. Time-lapse imaging has its own benefits, like low light exposure in relation to traditional morphology observation methods [63] and possibility of observing embryo inside incubator without moving it, which provides stable and uninterrupted conditions that could be beneficial for the final result [64].

Several statistical models were created to predict parameters like blastocyst stage development and quality. Morphokinetic algorithms that could predict successful implantation, biochemical or clinical pregnancy were described [60, 65, 66].

3.2 Embryo aneuploidy

Throughout the life a human body and their cells are affected by many negative life-style and external environment factors. Adding the aging and cellular senescence, it results in errors in chromosome segregation during meiosis I and II [67]. Due to the aging of the oocytes, these errors lead to increase of embryos with abnormal chromosome number. The reasons behind these chromosome segregation errors are due to many factors, like incorrect formation of bivalents, derogation of cohesins, sister kinetochores separation by large distances and incorrect attachment of spindle microtubules to kinetochores during meiosis. Despite the well-known fact of decreasing quality of oocytes with aging, many women in the modern age delay having their first child until later in their life [68].

One of the largest studies on the impact of age on the aneuploidy rates was performed in 2011. More than 20 000 oocytes were obtained from 2830 patients with an average age of 38.8 years, and their polar bodies were examined using FISH method. A study detected 20% of aneuploidies in women at the age of 35 that increased to the nearly 60% aneuploidy oocytes in women over 43 years of age. Of all the tested oocytes, almost half of them (46.8%) were aneuploid [69].

Detection and management of embryo anomalies that occur due to the age, poor quality oocytes or sperm abnormalities may be accomplished by performing preimplantation genetic testing (PGT) that allows to pick up and transfer blastocysts with normal genetic constitution.

The history of PGT goes back to 1989 when Handyside performed first pre-implantation genetic diagnostic (PGD) cases detecting a Y chromosome-specific region with PCR in case of X-linked adrenoleukodystrophy and X-linked mental retardation [70]. Defining embryo gender can complement to genetic testing of monogenic disorders linked to the sex chromosomes.

With time PGT underwent significant methodological changes, starting from the polar body testing and blastomere analysis to adapting trophectoderm biopsy with subsequent blastocyst freezing [71]. In early days the blastomeres were analyzed using FISH method for chromosomes X, Y, 18, 13, and 21 [72]. The analysis of more than a single cell has led to a more robust downstream molecular investigation [73]. Molecular genetic testing started as analysis of single loci by PCR method and grew to sophisticated single cell whole genome amplification [74]. Also, instead of PGT many groups have tried to develop algorithms to detect ploidy based on morphokinetic properties. There were several attempts to create such algorithms using time-lapse monitoring. The idea was based on assumption, that embryos display different cleavage dynamics depending on their genetic material, but this have not been fully proved [75, 76].

PGT consists of two main tests: PGT-M and PGT-A. PGT-M is a pre-implantation genetic testing of embryos for monogenic (or single gene) diseases. PGT-M is used on as a part of IVF process in couples with hereditary genetic disorders.

PGT-A (preimplantation genetic testing for aneuploidy) is a procedure that allows determining the chromosomal status of IVF embryos by screening all 23 pairs of human chromosomes including sex chromosomes. Many different methods are used, which includes array comparative genomic hybridization (aCGH), quantitative PCR (qPCR), single nucleotide polymorphism array (SNP array) and next-generation sequencing (high and low resolution) (NGS). The difference between those methods is in quantity of genomic amplification, ability to detect balanced or unbalanced translocations, partial aneuploidies, polyploidy, and mosaicism. For example, Array CGH, SNP array, and high resolution NGS utilize whole genome amplification of genomic DNA but at the same time can introduce an artifact. Quantitative PCR and low resolution NGS are not able to amplify the whole genome

and because of their low genomic coverage small deletions or duplications could not be detected [72].

In the PGT-A results embryos can be diagnosed three ways: as euploid with the normal number of chromosomes; as aneuploid with abnormal number of chromosomes, or mosaic - where 2 different cell lines are present within the same embryo (often one euploid cell line and one aneuploid cell line). Regarding mosaic embryos, it has been shown that mosaicism rates decrease with extended embryo culture. This could happen due to embryos ability to self-correct or because euploid cell lines predominate at later developmental stages [77].

However, the data on the ability of the PGT-A to improve the implantation and live birth rates in RIF patients is also still a controversial issue [36, 78–80].

4. Immunologic factors

4.1 Lymphocytes Th1/Th2 profile and Th17

T lymphocytes are types of immune cells that originate from bone marrow and mature in thymus cortex. One of those mature populations of lymphocytes are T-helpers, which express antigens CD4. CD4+ T cells are divided into two major types: T helper 1 (Th1) and T helper 2 (Th2) cells. Th1 and Th2 are characterized by cytokines that they secrete and are important in cellular and humoral immunity function. Th1 in general tends to be proinflammatory and secrete such cytokines as interferon- γ , TNF- α and interleukins (ILs) 1, 2, 6, 12, 15 providing help to other T cells and macrophages. Th2 on the other hand, cancel out the Th1 subpopulation and serve as anti-inflammatory agents that secrete ILs 4, 5, 10 and provide help to B cells, in the production of antibodies [81, 82].

During pregnancy, the milieu of Th cells in normal circumstances shifts towards the prevalence of Th2 subpopulation. It happens due to rise of Progesterone, which inhibits the secretion of Th1 cytokines. In immunologic profiles the levels of IL-4 and IL-10 goes up and levels of IL-2, TNF- α and interferon- γ goes down. When the child is born, he/she also has a Th2-dominant cytokine milieu, which quickly changes because of the microbial colonization [83]. If the milieu reverses towards the Th1 cell dominance, it could impact pregnancy by causing cytokine-triggered abortion due to thrombotic/inflammatory processes in maternal uteroplacental blood vessels.

It has been demonstrated on mice that injection of Th1 cytokines, i.e., TNF- α or IFN- γ mediates abortion, while the administration of TNF- α antagonists reduces the fetal loss [84]. However, Th1 prevalence in the peripheral blood during peri-implantation period is normal in healthy women, also, there is no correlation between cytokine expression and serum hormone levels, which makes screening tests difficult to use in predicting imbalance in future pregnancies [85, 86].

Liang et al. compared the ratios of pro- and anti-inflammatory cytokines IFN- γ /IL-4, IFN- γ /IL-10, IFN- γ /TGF- β 1, TNF- α /TGF- β 1 in the RIF patients and women with successful pregnancies. They discovered the shift towards pro-inflammation in RIF group [87]. Another group also indicated that without any difference in gestational age, the pro-inflammatory cytokine levels such as TNF α , IFN γ , are significantly higher in euploid miscarriages, than in healthy pregnant women at 10–14 weeks of pregnancy [88].

As the role of Th1/Th2 population gain more and more evidence of their relevance in fertility, the role of third population of cells like Th17 also gained attention. Th17 cells are types of T-helper lymphocytes that are characterized by pro-inflammatory cytokine IL-17 production. Signals such as transforming

growth factor beta (TGF- β), interleukin 6 (IL-6), interleukin 21 (IL-21) and interleukin 23 (IL-23) cause the Th17 formation in mice and humans while at the same time these signals inhibit regulatory T cell differentiation [89, 90]. IL-17 is a cytokine which promotes the expression of various mediators of inflammation while playing an important role in maintaining mucosal surfaces pathogen free. The loss of Th17 cell populations at mucosal surfaces is related to chronic inflammation and microbial translocation. It has been demonstrated that the levels of Th17 cells in peripheral blood lymphocytes do not change in women with normal pregnancy [91] but proportion of Th17 cells in the peripheral blood and decidua significantly increases in unexplained recurrent spontaneous abortion patients [92, 93].

4.2 TNF-a

Overproduction of TNF-a could have toxic effect of pregnancy despite its necessity to assure endometrial receptivity. By analyzing cytokine profile of 210 women undergoing IVF with the endometrial secretion analysis technique, higher TNF-a and IL-1b levels have been detected in patients with the previous history of implantation failure that achieved clinical pregnancy [94]. This could emphasize, that proinflammatory cytokines are also needed for successful transfer, pregnancy to occur.

4.3 Regulatory T cells

Regulatory T cells, formally known as suppressor T cells or Tregs express the CD4, FOXP3, and CD25 biomarkers and develop from the same lineage as T-helpers [95]. These cells are best known for their function to generally suppress Th1- and Th17-mediated immunity, or in other words - to suppress autoreactive and alloreactive immune responses, thereby preventing autoimmune diseases and allograft rejection [96]. These cells also modulate the immune system and downregulate induction and proliferation of effector T cells (CD8+). Tregs are involved in the regulation of local maternal tolerance towards the fetus, their concentration increases within 2 days of conception in normal human pregnancy [97]. Expression of IL-10 and TGF-b by Tregs and its suppression potential is significantly reduced in patients with unexplained recurrent spontaneous miscarriage in comparison to fertile patients [98]. Tregs also regulate the CD8(+) T cell differentiation by significantly reducing the expression of perforin and granzyme B in the decidua compared to peripheral blood EM CD8(+) T cells, which may also play a crucial role in establishing the maternal immune tolerance cells [99]. It has been demonstrated that patients with recurrent miscarriages and cellular immune imbalance could be treated with intravenous immunoglobulin G given it increases the of Th17 and Foxp3(+) Treg cell numbers [100].

4.4 Natural killers (NK) and uterine NK (uNK)

Parents wishing for a pregnancy are not tissue-matched, therefore, a mother's immune system has to be suppressed. NK cells seem to play an important role in this process [101].

Natural killer cells, or NK cells, are a type of cytotoxic lymphocytes. In the uterus there are a special type of Natural Killers that consists of two main subsets: CD56 + CD16+ cells (10% of uNK) with dim phenotype, and CD56 + CD16-cells

(90% of uNK) with bright phenotype [102]. The bright type that should be dominant cell subset, has low cytotoxic ability and are potent in secreting the cytokines [101].

IL-15 and IL-18 are involved in the maturation of uNK. As the potential markers of uNK activity, levels of IL-15 and IL-18 are positively correlated with uNK levels in patients with implantation failure [103]. uNK cells increase in numbers from about 70% up to 83.2% of the uterine leukocytes during the mid- to late luteal phase, and first trimester of pregnancy [104, 105]. In some studies, it has been established that uterine NK play a role in decidual vascularization. This elevation of uNK CD56 cells density could contribute to increased angiogenesis in pre-implantation period leading to reduced uterine artery resistance to blood flow, endometrial oedema and as a result to implantation failure [106, 107]. Abnormal decidual vascularization and the increase in angiogenic factors contribute to the development of miscarriages and implantation failures.

4.5 Altered expression of associated molecules

4.5.1 Prostaglandins

Prostaglandins are group of physiologically active lipid compounds called eicosanoids. Prostaglandins are synthesized from Arachidonic acid by the action of cyclooxygenase (COX) isoenzymes [108]. Their function is to sustain homeostasis, mediate pathogenic mechanisms by stimulating a reaction in one tissue (inflammatory response) and inhibiting the same reaction in another tissue. The type of the receptor to which the prostaglandins bind determines these reactions.

Decidual cells also secrete prostaglandins that latter interacts in complex reactions with other cytokines, growth factors and hormones like progesterone, prolactin, relaxin.

Achache et al. suggested that decreased prostaglandin synthesis might be a key factor in altered endometrial receptivity, therefore plays a role in implantation failure. Levels of cytosolic phospholipase A2 (cPLA2a) and COX-2 that was measured by PCR and Western blot tests were found to be decreased in patients with RIF in comparison to the fertile group. It was suggested that it might be detrimental to implantation only when both enzymes are missing. Interestingly, in response to the decreased function and presence of these enzymes, secretory phospholipase A2 (sPLA2-IIA) was overexpressed. This overexpression most likely is a form of compensation to maintain release of arachidonic acid [109].

Another data on RIF patients show that they express defective endometrial PG on the days 21–24 of the cycle. It has been estimated at both mRNA and protein level that already mentioned enzymes such as cyclooxygenase-2 (COX-2), secretory phospholipase A2 group (sPLA2-IIA, sPLA2-V, sPLA2-IB), and other molecules like glypican-1, PG-E synthase, PG-E receptors, and lysophosphatidic acid receptor 3 (LPA3), play an important role in PG synthesis and are found in very low concentrations in 85% of RIF patients [109].

4.5.2 HOX-a and E-cadherin

Certain type of transcription factors, such as HOXA-10 and E-Cadherin, could also play the potential role in the implantation process. HOXA-10 and E-Cadherin are localized in the glandular epithelium cells of endometrium. Specifically, HOXA-10 in the nuclei of stroma cells and E-Cadherin the cytoplasm of glandular

epithelium cells. Regarding HOXA-10 and E-cadherin expression, positive correlation was established between significantly reduced levels of HOXA-10 and E-cadherin expression in women with the history RIF, as compared with a control group [110].

4.5.3 Leukemia inhibitory factor (LIF)

LIF primarily was identified as macrophage differentiation inductor of the myeloid leukemia M1 cell line [111]. Later LIF started to be considered as the first cytokine that is shown to play critical role in mice blastocyst development and implantation [112]. The other functions of LIF included proliferation, differentiation and cell survival [113]. Given the fact that LIFs mRNA is expressed during days 18–28 of the menstrual cycle in the endometrium of the fertile women, it also suggests it has a role in human implantation [114]. LIF expression is regulated by Progesterone. Treating women with the progesterone receptor antagonist immediately after ovulation, it reduces immunoreactivity of LIF at the expected time of implantation [115].

Evidence for LIF role in human fertility was described several authors showing that low concentrations of LIF in the uterine flushing fluid at day 26 may be a good predictor of successful implantation, while at the same time endometrial explants from infertile women and women with recurrent miscarriages secrete less LIF than those from fertile women [116–119].

4.5.4 Apolipoprotein A-I

From the proteomic analysis of the endometrial biopsies, a new molecule was found that could be a potential predictor of the endometriosis and even of the RIF patients.

Apolipoprotein A-I (Apo A-I) was identified as an anti-implantation protein, that is secreted by differentiating endometrium. It seems that higher expression of Apo A-I can be found in ectopic secretory endometrium in patients with endometriosis. This statement implies that dysregulation of certain molecule secretion might be a significant factor in pathogenesis of endometriosis and a crucial point for RIF [120]. But more data on this “fingerprint” is needed to apply it to the diagnostics.

4.6 Antiphospholipid antibodies (APL)

Antiphospholipid syndrome (APS) is an autoimmune condition that is associated with thrombosis and morbidity in pregnancy [121]. The pathogenic pathway by which whose conditions occur are not yet fully understood but it is known that mechanisms may be heterogeneous. The main Antiphospholipid Antibodies (aPLs) that is found in APS are lupus anticoagulant (LA), anticardiolipin antibodies (aCLs) and anti- β 2glycoprotein I antibodies (a β 2GPI) [122].

APS is diagnosed if at least one of three antiphospholipid antibodies are detected on two or more occasions within a 12-weeks interval, and is associated with a clinical condition such as thrombosis or morbidity in pregnancy. Morbidity in pregnancy includes preterm delivery due to eclampsia, preeclampsia, unexplained stillbirths at ≥ 10 weeks of gestation, or placental insufficiency, and three or more consecutive miscarriages [123]. Recurrent early miscarriage is one of antiphospholipid syndrome (APS) obstetrical features with the incidence of aPLs in 15–20% of the patients with recurrent miscarriages [122]. However, the link between APS and RIF should be further investigated.

5. Other factors

5.1 Body mass index (BMI)

Obesity is defined as BMI equal to or more than 30. According to the WHO, more than 1.9 billion adults of the world population are suffering from the extra body mass (BMI >25), and more than 650 million are obese [124].

A significantly lower ongoing pregnancy rate and implantation rate in obese women have been reported [125, 126]. The implantation rate, pregnancy and live birth rate are shown to be lower in obese women with the tendency to progressively go down with each unit of BMI (kilograms per square meter) [127]. It has been also found that higher BMI is associated with lower clinical pregnancy rates especially in women under age 35 using their own oocytes [128].

5.2 Ovarian response to stimulation

The goal of ovarian stimulation is the collection of multiple dominant follicles in an effort to compensate for the inefficiencies of embryology culture, embryo selection, thus improving chances for successful conception in IVF [129]. The definition of poor ovarian response (POR) should be understood as an essential inability of woman's ovaries to properly react to the selected stimulation [130]. At least two of the following three features must be present for the POR to be diagnosed: 1) advanced maternal age (≥ 40 years), or any other risk factor for POR; 2) a previous POR (≤ 3 oocytes with a conventional stimulation protocol); 3) an abnormal ovarian reserve test (antral follicle count: 5–7 follicles, or Anti-Mullerian hormone 0.5–1.1 ng/ml) [131–133].

There are many risk factors that may cause poor ovarian response: short menstrual cycle, single ovary, ovarian cystectomy, smoking, unexplained infertility, previous chemotherapy and/or radiotherapy treatment, family history of premature menopause, pelvic infection, etc. Further studies are needed to elucidate the role of these factors in RIF.

5.3 Male factor

Many studies have emphasized the role of sperm DNA integrity on the fertility of a couple, also reporting the relationship of the increased sperm DNA damage and pregnancy loss after IVF and ICSI [134]. However, some studies have failed to support a hypothesis that sperm DNA integrity is an important factor in RIF [135], therefore, also this important question needs to be further investigated.

6. Conclusion

We have described that recurrent implantation failure in IVF cycles depends on the interplay of many factors - female factors (different aspects of the uterine health), embryo factors (embryo quality and aneuploidy), possibly male factors (sperm DNA integrity), immunological factors, and probably many more. More investigations are needed before the clinicians can be clearly advised what diagnostic and treatment approaches must be implemented in the cases of RIF.

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