Immunology and implantation failure

Immunologie et absence d’implantation

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Abstract. The role of autoimmunity and thrombophilia in recurrent implantation failure (RIF) is one of the most controversial issues in assisted reproduction. Implantation is a delicate equilibrium between embryo quality, endometrial receptivity, and the efficiency of the embryo transfer technique. Although results of egg donation treatments suggest that the embryo is the most important factor in this equilibrium, there might be other factors that can contribute to implantation failure, such as the endometrium, the efficiency of the embryo transfer or maternal tolerance to pregnancy. However, we do not know which group of patients may benefit from immune therapy for RIF, and empiric approach seems to be useless. This is in line with recent guidelines by the Royal College of Obstetricians and Gynaecologists and the American Society for Reproductive Medicine. In order to determine which women might benefit from manipulation of the maternal immune system, further research is needed.

Key words: recurrent implantation failure, immune therapy, thrombophilia

Résumé. Le rôle des processus auto-immuns et des thrombophilies dans la pathogenèse des échecs d’implantation répétitifs est l’un des sujets les plus controversés au sein de la procréation médicalement assistée. L’implantation est le résultat d’un équilibre délicat entre la qualité embryonnaire, la réceptivité endométriale et l’efficacité de la technique du transfert embryonnaire. Bien que les résultats des traitements de don d’ovocytes indiquent que c’est l’embryon qui est le facteur le plus important de cet équilibre, il peut également y avoir d’autres facteurs qui peuvent contribuer à l’absence d’implantation, y compris l’endomètre, la technique du transfert ou la tolérance immunitaire maternelle face à la grossesse. Néanmoins, il reste à savoir quel est le groupe des patients pouvant bénéficier d’une thérapie immunitaire contre les échecs répétitifs d’implantation, tandis qu’un abord empirique, selon les directives récentes de diverses associations scientifiques, ne semble pas utile.

Mots-clés : échec d’implantation répétitif, thérapie immunitaire, thrombophilie

Among all the different topics that are dealt with nowadays in assisted reproduction, one of the most controversial issues is the role of autoimmunity and thrombophilia in recurrent implantation failure (RIF). In recent decades, substantial progress has been made in simplifying the diagnostic approach of the couple, understanding folliculogenesis, controlling ovarian stimulation, facilitating egg retrieval and embryo transfer, as well as culturing embryos up to the blastocyst stage. However, there is still a lot to learn about endometrial receptivity and about the embryos beyond their morphology. This would help us to understand better the process of implantation and the mechanisms underlying RIF.

Implantation is a delicate equilibrium between embryo quality, endometrial receptivity, and the efficiency of the embryo transfer technique. One of the ways to define RIF is by way of life table analyses. In a recent paper published by Garrido et al. [1], cumulative live birth rate (LBR) was analyzed according to the number of transferred embryos in IVF cycles. With the transfer of 5 embryos, a 52% cumulative LBR was reached, whereas after having transferred 10 embryos, cumulative LBR was found to be 69%, and after 15 embryos, 79% of patients reached live birth (figure 1). But what happens with the rest of cases? Is it the embryo that despite looking “good enough” has genetic or epigenetic alterations that inhibit its implantation or is it the endometrial receptivity or the maternal immune tolerance? In fact, by repeating IVF cycles, most of the couples get pregnant, while the only thing that changes is the composition of the embryo, which is then transferred to the same uterus – in the same mother.
This might make us think that it is the embryo that weighs more in this equilibrium. If we look at numbers that come from egg donation cycles, most of the patients perform very well: based on the results published by our group [2], cumulative LBR can be as high as 97% after transferring 15 embryos derived from egg donation (Figure 2), which means that the risk of implantation failure is much lower with good quality embryos.

However, we cannot ask embryos to do more than what they can do. There might be other factors that contribute to implantation failure, such as the endometrium, the efficiency of the embryo transfer or the maternal toleration to pregnancy. Repeated implantation failures make both patients and their physicians desperate and eager to try many things, so one can easily end up testing and treating alterations without sufficient scientific evidence. In most cases, anti-thyroid antibodies, NK cells, antiphospholipid antibodies and thrombophilic disorders are tested.

Basically, we can define autoimmunity as the failure of the body to recognize its own constituent parts as “self”, which triggers an immune response against its cells. Whereas a high level of autoimmunity is unhealthy (like in the case of celiac disease, type 1 diabetes mellitus, or systemic lupus erythematosus), a low level of autoimmune reactions might be beneficial, such as in the recognition of neoplastic cells or in the rapid response at the early stages of infection.

As far as thyroid autoimmunity is concerned, several retrospective and observational studies, as well as underpowered trials have been published. In a recent metaanalysis of these studies [3], euthyroid, subfertile women with thyroid autoimmunity showed similar LBRs as control women, which means that we probably should not be testing thyroid autoimmunity in euthyroid women except under a research setting.

As opposed to an earlier theory defining the embryo/fetus as a “foreign body” totally compatible with the mother [4], nowadays we consider pregnancy as a cooperation venture between fetal antigens and maternal immune cells [5]. In this context, the mechanism of action of immunomodulating agents, such as steroids, intravenous immunoglobulin (IVIG) or anti-TNF-alpha, involves the modulation of complement activation, the suppression of idiotypic antibodies, the saturation of Fc receptors on macrophages, and the suppression of various inflammatory mediators, including cytokines, chemokines, and metalloproteinases.

The study of natural killer (NK) cells is one of the most controversial areas in reproductive autoimmunity. There are two types of NK cells, uterine and peripheral. Both of them are part of the immune system and both express surface antigen CD56. However, phenotypically
and functionally they are very different from each other. Less than 10% of peripheral NK (pNK) cells resemble uterine NK (uNK) cells. Ninety per cent of them are CD56\textsuperscript{dim} and CD16+, and they exert significant cytotoxic activity, whereas uNK cells, which appear in the midsecretory phase, have very little cytotoxic activity, 80% of them are CD56\textsuperscript{high} and CD16−, and they express KIR, ILT-2, NK G2, as well as HLA-C, E, and G. Therefore, these two types of NK cells are two separate entities.

The association between pNK cells and fertility problems has been the subject of several studies in the last twenty years. There are several papers that conclude that there is an association with recurrent miscarriage [6-10] and with infertility [8, 11, 12], whereas more recent studies have not found any correlation [13-16]. Similarly, some studies have demonstrated an association between uNK cell number or activity and recurrent miscarriage [17, 18] or infertility [19], whereas other studies claimed just the opposite [20-22]. Apart from a tremendous inconsistency among these papers – their methodology not being of equally high standards –, the causative role of NK cells in reproductive problems is also questionable.

In a recent metaanalysis, summarizing all relevant studies on uterine and peripheral NK cells with a total of almost 800 cases [23], no difference was found in the odds for implantation failure either in relationship with pNK cell numbers or their activity. The authors therefore concluded that the prognostic value of measuring pNK and uNK cell number or activity remains uncertain. The value of an abnormal test for pNK cells is unknown. Their levels and activities may change with stress or even during controlled ovarian hyperstimulation. Also, there is no consensus on what an abnormal NK cell test result is. Most of the groups consider 12% as the upper limit of normality, but there is huge variation between 5 and 29%, without any clear explanation as to the choice of the specific cut-off. Important differences can be observed in the analysis and interpretation among the studies included, and they also show considerable heterogeneity.

Although there is no clinical evidence, is there a biological plausibility for the role of NK cells in infertility? Uterine NK cells are abundant at implantation [24], and they interact with extravillous trophoblast cells [25]. The uNK cell population in decidual tissue from normal pregnancies is different from that found in pregnancies ending in miscarriage [26]. They regulate angiogenesis [27, 28], and there are trophoblasts that express antigens recognized by uNK cells [29].

As far as the use of IVIG in patients with RIF is concerned, a randomized trial showed no improvement in pregnancy outcomes as compared to placebo [30]. Similarly, no significant differences were found after IVIG treatment in patients with recurrent miscarriage, neither in separate, small-scale studies, nor in a recent metaanalysis summarizing their data [31]. Another metaanalysis published this year did not find any evidence to support the routine use of adjuvant therapies for women with elevated NK cells undergoing assisted reproduction techniques in order to improve LBR [32].

Thrombophilia can be characterized as hypercoagulability or a prothrombotic state, which means that it increases the risk for thrombosis. This, however, will only develop in the presence of other risk factors, such as high estradiol concentrations, pregnancy or obesity. Even though a significant proportion of the population has a detectable abnormality, most of them are asymptomatic. The usual panel of thrombophilia tests includes anti-cardiolipin antibodies, lupus anticoagulant, antithrombin III deficiency, activated protein C resistance (and, if positive, genetic testing for the Leiden mutation of factor V), protein C and S activity, plasma homocysteine levels, the screening for variants C677T and A1298C of the MTHFR gene, as well as mutation G20210A of the prothrombin gene.

Prevalence studies show no difference in outcomes of IVF cycles between carriers and non-carriers of the factor V Leiden mutation or of prothrombin mutation G20210A [33]. A recent metaanalysis seemed to find significant differences in assisted reproductive treatment failure between carriers and non-carriers of the factor V Leiden mutation, but this effect was only seen in case-control studies – cohort studies did not confirm this finding. No significant differences were found in relation to prothrombin mutation G20210A, MTHFR variants, or deficient protein C, protein S or antithrombin III activity. RIF did, however, show a higher prevalence in the presence of antiphospholipid antibodies [34].

As far as empirical treatment of unexplained recurrent miscarriage with aspirin with or without low-molecular-weight heparin (LMWH) is concerned, an elegant randomized controlled study showed no difference in LBR whether or not patients were treated by either aspirin alone or combined with nadroparin, as compared to placebo [35].

When talking about anticoagulant or immunomodulating therapies, treatment risks need also be kept in mind. Treatment with LMWH increases the risk of thrombocytopenia and bleeding, while corticosteroids can cause fluid retention, hypertension, mood swings, weight gain, and they may increase the risk of infections, gestational diabetes, and developmental abnormalities in the fetus, such as cleft palate, if their administration is continued through the pregnancy. IVIG is a human derive, which has its own risks. Apart from causing headaches, dermatitis or pulmonary edema, it may trigger anaphylactic reactions, hepatitis, acute renal failure, deep venous thrombosis, aseptic meningitis, and an increased risk of diabetes in the newborn.

We clearly do not know which group of patients (if any) may benefit from anticoagulant or immune therapy for...
RIF. Most of these women show very good outcome after appropriate embryo selection or oocyte donation. There may be a subpopulation of patients with “unexplained” RIF due to some underlying autoimmune disease that we are unable to diagnose today, but empiric approach seems to be useless. This is also in line with recent guidelines by the Royal College of Obstetricians and Gynaecologists, which states that “this remains a research field and testing for uNK cells should not be offered routinely in the investigation of recurrent miscarriage” [36], as well as those by the American Society for Reproductive Medicine, according to which treatments with no proven benefit include leukocyte immunization and IVIG therapy [37].

In conclusion, most women with RIF are eager to try any form of treatment. There could be a potential for anticoagulant or immune therapy in an adequately screened group of patients. However, in order to determine which women might benefit from manipulation of the maternal immune system, further research is needed. There is little evidence to support any particular test or immunomodulatory treatment in couples with RIF. Therefore, for the meantime, they should be restricted to formal research studies.

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References


