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A Review on Controversies about the Role of Immune and Inflammatory Systems in Implantation Process and Durability of Pregnancy

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Abstract

Among the controversies about reproduction sciences, role of immune and inflammatory systems in implantation process and durability of pregnancy is a hot topic both in natural pregnancies resulting in spontaneous abortion and success rate of assisted reproductive technologies (ARTs). So we intend to represent and report our insights, reasoning and suggestions. This systematic and critical review includes investigation of scientific databases and tracing the citations. Our findings falls into three categories: role of immune system and leukocytes, vascular remodeling and histopathology of endometrium, role of inflammation in implantation process. According to the categories above, the immune and inflammatory systems not only are not harmful by itself, but also are necessary for spiral artery remodeling, implantation process and durability of pregnancy. At the end of the review it is notified and suggested that using a valid protocol is necessary for infertility clinical centers. The protocol mainly consists of human leukocyte antigen (HLA) C and G typing and also killer-cell immunoglobulin-like receptor (KIR) typing.

Keywords: Implantation, Infertility, Pregnancy maintenance

Introduction

Among the controversies in reproduction science, role of immune and inflammatory systems in implantation process and durability of pregnancy is a hot topic nowadays. Some researchers and practitioners believe that administration of drugs such as prednisolone, aspirin or heparin can improve success rate of implantation and pregnancy through inhibiting coagulation, inflammatory and immune systems; while others believe that such systems not only do not have negative effect on rejecting blastocyst and embryo by itself but also are necessary for implantation and remodeling of uterine spiral arteries through secreting adhesive and angiogenic factors. Such controversies are proposed both in natural pregnancies resulting in spontaneous abortion as well as in successful and/or unsuccessful assisted reproductive technologies (ARTs). Recurrent spontaneous abortion (RSA) is defined as having 3 or more history of abortion (1). About the other issue, some researchers and practitioners are grumbling of low success rate of ARTs like in vitro fertilization (IVF). Infertility is a problem for 10%-15% of couples; most of which – but not all – require ART (2). The average fertilization rate of ART in previous researches has been reported about 30% (3) and this report is for 1998. It has been claimed in an article published in 2015 that this rate has improved in the recent decade (4). Even though at the first glance this rate seems low, but the interesting point is that in the claim of a valid reference (5) the chance of natural pregnancy for each intercourse per month does not go up more than 33%. Also this number has been reported in some studies up to 40% (6). Thus, we should not be grumbling of the low fertilization rate of ARTs. Finally we intend to represent and report our insights, reasoning and suggestions in the present review.

Methods

This systematic and critical review includes investigation of scientific data bases and tracing the citations and the references of the articles in order to find the root of the truth. Through this wisdom, we showed the mere that a content proposed in an indexed article is not a reason that this content is valid and able to be cited. Indeed, the researcher should regard and notice the reasoning, citations and protocol of his/her references during the literature review. This is the benefit point of systematic review rather than holistic and narrative reviews. Since some data were few, the time period of publications was not important to us.

As the searching strategy, we used the Web of Science Core Collection and Google Scholar for finding articles other than ISI-WOS and the tracing mentioned in the above paragraph.

Results and Discussion

Role of Immune System and Leukocytes

In 2015, Ghafourian et al (1) by measuring the level of natural killer-cells (NKs) in blood of the RSA and the control group concluded that RSA patients in normal (non-preg-

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nant) conditions have significantly higher level of NKs. They accounted the high level of peripheral blood NKs as a risk factor for RSA which needs to be investigated further in their belief.

NKs play the first immune response of body and are in near contact with placenta that contributes as 5%-12% of the human total leukocytes (1), 40% of uterine stroma leukocytes along with macrophages (7) and 70%-90% of uterine leukocytes during implantation (1). NKs in peripheral blood are mainly $CD16^{+}CD56^{dim}$ and uterine NKs (uNKs) are mainly $CD16^{-}CD56^{bright}$ (1). They, also by citing to Muzzio et al (8) considered a dual effect for B lymphocytes; protecting from pregnancy by secreting antibodies and causing pregnancy complications by secreting autoantibodies.

Muzzio et al (8) along with reminiscing from immune system as a "fascinating phenomenon" announced that it can simultaneously protect the embryo and the mother against external pathogens. They cited from Kolb et al (9) that cytotoxic effects of maternal lymphocytes caused protection of cultured trophoblasts and ejecting of IgG from maternal serum was associated with decrease in the protecting effect. Borel et al reported (10) that antibody titer in serum of pregnant women was higher than non-pregnant women as another study (11) proved that patients with RSA have lower antibodies compared with the other women. Citing these contents as a positive approach to role of immune system by Muzzio et al – which is cited by Ghafourian et al (as a negative approach) - is notable. Progesterone-induced blocking factors of placenta can also induce production of antibodies from B cells (12). About the autoantibodies that could result in pregnancy complications, anti-phospholipid antibodies can cause induction of lupus anti-coagulant and anti-phospholipid syndrome. These autoantibodies result in hyper-coagulopathy that in addition to causing infertility, also have a risk of arterial or venous thrombosis for the mothers (13) which seems to be able to treated with aspirin or prednisolone.

As returning to Ghafourian et al paper, they are of the conviction that the role of peripheral and endometrial leukocytes in the outcome of pregnancy in patients with RSA is still controversial. They have announced that according to abundant population of NKs in decidua they must per se perform physiologic and not pathologic functions. They also cited from Karami et al (14) that no significant correlation is observed between increase of $CD56^{bright}$ and RSA patients. Of course increase of $CD56^{dim}$ in RSA was non-controversial and successive in previous original studies. Karami et al believe that in spite of lack of expression of usual human leukocyte antigen (HLA) class I (A and B types) by trophoblast cells, they are not attacked by uNKs and the mechanism of the trophoblast escape is still unknown. Recently Sachs in a commentary announced that empirical approach to role of NKs is not enough and researchers from different fields should work more closely together with a scientific approach to provide the best care for patients (15).

In the Karami et al study, not allowing patients for regional sampling of endometrium, led to lack of investigation of uNKs; because this work required having endometrial biopsy in the secretory phase of menstrual cycle. This limitation was also the reason of the lack of investigation of the uNKs ($CD16^{-}CD56^{bright}$) in other studies.

Among the little studies on human uNKs, we can name the study of Lachapelle et al in 1996 (16). In the study of Lachapelle et al, in RSA group, endometrial $CD8^{+}$ T cells had significantly decreased and the proportion CD4/CD8 had increased. On the other hand, the number of $CD20^{+}$ B cells was significantly increased. The main and interesting result of this study was that $CD16^{-}CD56^{bright}$ NKs had significantly decreased in RSA group. So we can conclude that although based on the successive and undeniable resources, $CD16^{+}CD56^{dim}$ NKs are further in peripheral blood of RSA group, but inverse, about uNKs we had decreasing rate. It shows that the hypothesis of adverse effect of uNKs (in comparison to blood NKs) on saving blastocyst and embryo and also administration of aspirin to inhibit them is still under question; why it seems that uNKs have per se protective effect on blastocyst and embryo.

Also the study of Lachapelle et al has it that $CD16^{-}CD56^{bright}$ NKs secrete cytokines including some colony stimulating factors that may help the promotion of placental growth. Because of this dual and paradoxical effect of uNKs and blood NKs it seems that imbalance between the $CD16^{-}CD56^{bright}$ and the $CD16^{+}CD56^{dim}$ is effective on RSA.

In addition to CD56, CD16 marker is remarkable. In contrast to CD56, CD16 is more an effective agent rather than being a diagnostic marker; in other words, CD16 is the weapon of NKs for antibody dependent cell-mediated cytotoxicity (ADCC) (17). Since uNKs are mainly $CD16^{-}$ they are called as immune-regulatory NKs (against cytotoxic NKs); and since mostly $CD56^{dim}$ is along with the presence of CD16 and $CD56^{bright}$ and absence of CD16, the dim and bright types of NKs are respectively called as cytotoxic and immune-regulatory NKs (18).

As we mentioned before, HLA genes are the most polymorphic loci in the human genome that is very useful in anthropological studies (19) as well as infertility screenings. In addition to the HLAs, about 14 types of killer-cell immunoglobulin-like receptor (KIR) have been discovered so far on the surface of NKs. Rather in which KIR bounds to which HLA (type C or G), NK performs different functions that most of them are protective for maintenance of pregnancy (20,21).

Another role in immune system is that mesangial stem cells in menstrual blood can modulate the activity of immune cells such as B cells, T cells and NKs (22-24). So the menstruation of women as a natural process shows the immune system as the fascinating phenomenon. Another paper on mice (25) showed that the number of dendritic cells in early pregnancy is high rather than intermediate as in late days of pregnancy or non-pregnant conditions. It shows the natural and vital role of immune system in early pregnancy.

The interaction between embryo and maternal tissue de-

pends on hormones, adhesive molecules and growth factors (6). Most physiologic immune responses start with proceeding of natural intercourse because of a lot of beta growth factors and prostaglandin E volume in semen that enable it to induce T-regulatory cells (26). Thus, inhibiting immune system as a protocol is throwing the body out with bath water. For example, uNKs, T-helper-1 and dendritic cells are not only necessary for balance of immune system but also for vascular remodeling and decidual development (26).

Even nowadays most roles of NKs are unknown. The immune-tolerance is mainly handled by uNKs. The final mechanism seems to be T cell immunoglobulin and mucin domain-containing protein 3 (Tim-3), a newly discovered molecule which is highly expressed on cell surface of uNKs. Abnormal Tim-3 level might be associated with RSA (27). As described by Wang et al. Tim-3 promotes T-helper balance (28).

Vascular Remodeling and Histopathology of Endometrium

Uterine spiral arteries perform an important function in supplying nutrients to uterus and in order to gain this aim, these arteries are supposed to be remodeled; hence they get physiologic dilating that otherwise leads to pre-eclampsia (29) – a pregnancy specific syndrome in which a variety of immune factors are involved (30).

Uterine vascular remodeling is one of the controversial issues in histology and embryology that approximately in all related articles it is known as an unknown process. So the final accepted idea in most papers like Ashton et al (31) is that for sufficient blood supplying of embryo it is necessary that uterine arteries should "revert", in which its endothelial cells are replaced by extra villous trophoblast (EVT), surrounding smooth muscles and fibrinoid (Figure 1), (29,31-33).

In the first trimester of pregnancy, decidual spiral arteries change from narrow position into dilated form with lack

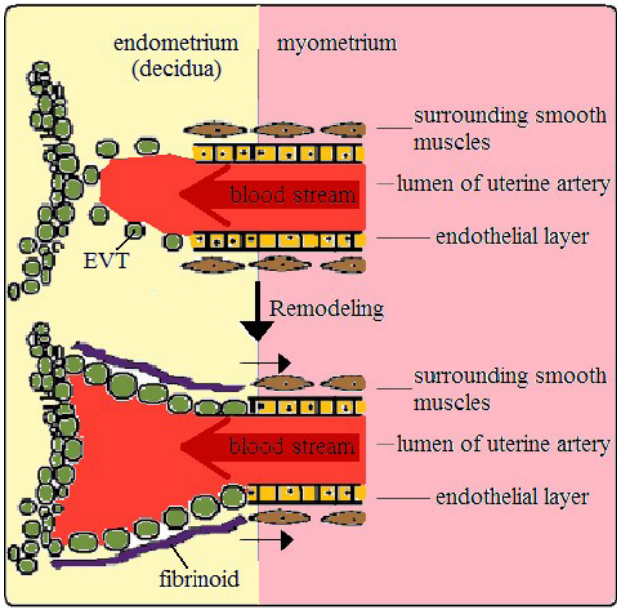

Figure 1. Remodeling Process of Uterine Spiral Arteries.

of maternal vasomotor control. These changes are performed for better blood supplying that if failed leads to pregnancy complications (7).

A controversial point is how to remodel. We found that in this going back of spiral arteries we have apoptosis in endothelial layer (29); whereas in muscular layer we have cell migration and no apoptosis was observed (32). The mentioned apoptosis is triggered by leukocytes dealing with remodeling (like NKs, etc.) (7).

In mice with impaired NKs it has been observed by Pijnenborg et al that the muscular layer of spiral arteries remained intact (29). So it can be concluded that existence of NKs is necessary for migration of muscular cells and inhibition of them may cause complications. Also he believed that uNKs play two roles; direct impact on vascular remodeling and regulatory impact on trophoblast invading. Also, it has been observed in golden hamster (34) that uNKs are involved with vascular remodeling during implantation while they invade arterial wall before invading trophoblasts. uNKs gathered in the location of remodeling, cause vascular dilation and angiogenesis by secreting vascular endothelial growth factor (VEGF) and interleukin (IL)-8 (34). For this reason, progesterone therapy is done to improve expression of VEGF receptor in endometrium at implantation time (35). Although Clifford et al (36) observed an increased number of NKs in decidua of women with RSA, they investigated the total uterine $CD56^{+}$ cells which consists of both bright and dim forms of CD56.

Role of Inflammation in Implantation

Over 75% of pregnancy failures are associated with implantation failure (37). Of the controversial issues is whether inflammation is good or bad in the implantation success. For this reason some previous studies proposed that administration of low dose aspirin, heparin and prednisolone to increase the success rate of ARTs is an effective method (38); because coagulation process and the coagulation factors are involved in pregnancy loss as described by Jeddi-Tehrani et al. (39). The more interesting point is that no significant correlation were observed in approximately half of these studies. It can never be denied that pathologic and unusual inflammation impedes the success pregnancy, but by itself inflammation is a physiologic phenomenon that is necessary for pregnancy maintenance.

About administration of low dose aspirin as an adjuvant treatment of infertility, there are controversial approaches and contradictory findings. For instance, Akhtar et al in 2013 as opposed to aspirin administration, announced directly in their study entitled "Aspirin and heparin as adjuvants during IVF do not improve live birth rates in unexplained implantation failure" (40). After a year, Gizzo as a defender of aspirin administration takes success less of such researches into account as lack of suitable protocol (41); but even in his research, numbers of good-quality embryos were decreased in group of low dose aspirin administration. Another research (42) announced that inhibition of NKs – with aspirin or prednisolone - results

in better interface between embryo and maternal tissue as Boroujeni et al announced (43). On the hypothesis of Haapsamo et al (44), administration of low dose aspirin can improve vascular remodeling; but it's to be noted that inhibition of immune system is somehow throwing the body out with bath water; as a valid paper in *Journal of Reproductive Immunology* in 2015 announced that uNKs can help durability and remodeling of uterine arteries (45).

Moini et al in 2007 (46) in a research – with a similar protocol to Akhtar's (100 mg aspirin daily for 12 weeks after IVF performing) – in spite of the defending approach to aspirin administration, reached no significant correlation. Of course unlike Akhtar and like Gizzo, who consider failure of their hypothesis because of unsuitable protocol. This reasoning is correct; because 12 week administration of such high bio-availability drug inhibits natural inflammation and immune system of patients in the sensitive period of placentation. Of course even Gizzo's protocol was not responsive and for this reason in our previous letter we suggested the administration merely at ovulation period (38). Thanks to the up-regulatory effects of aspirin on ovary and ovarian hormones (43). But because of histopathology sequels of ovulation stimulation (47,48) it is still controversial.

Apoptosis (triggered by IFN-gamma) in turn like inflammation is a physiologic phenomenon by itself and seems necessary for vascular remodeling and implantation window formation (49). For example, the mentioned EVTs induces apoptosis through interaction of Fas/FasL (31). Of course in pathologic conditions the very apoptosis could be a reason of abortion (50), like apoptosis secondary to toxoplasma infection (51).

Since in the word of Longman's Embryology, L-selectin is responsible for primary adhesion of blastocyste to endometrium (52) and on the other hand non-steroid anti-inflammatory drugs (NSAID) in general and aspirin in particular can inhibit L-selectin (53), aspirin administration even at early implantation might be against logic. The other extra-cellular molecules such as Laminin (54) and Fibronectin in the word of Longman are involved in the adhesion (52), in which aspirin is respectively non effective on the first one (55) and inhibitor of the second one (56). "Laminin receptor 1" may also have an important role in human reproduction. Its decreased expression has also been linked to shallow trophoblastic invasion which may lead to pre-eclampsia (54,57,58). Of course such molecules have variety of kinds and the mentioned references (53,55,56) are not merely related to endometrium and it's upon us to study further. Also L-selectin is abundant on cell surface of $CD56^{bright}$ NKs (19); so inhibiting these NKs as a therapeutic protocol by aspirin needs to be investigated further.

The inflammatory response of T-helper-1 cells and T-helper-2 cells are respectively necessary for induction of implantation and pregnancy maintenance (59). Another research showed that in uterine stroma cells of healthy women, level of cytokines such as IL-6,8 and transforming growth factor (TGF)-beta were upper than in women with implantation failure history (6). In study of Rajaei et al (6) because of some impediments, investigation on Interferon (IFN)-gamma was not performed; but another study in 2015 proved its impact (60). Thus, it seems that such pre-inflammatory cytokines are necessary for implantation success. The impact of tumor necrosis factor (TNF)-alpha is also reported (61).

As described by Murphy et al in a high quality review (49), IFN-gamma (secreted mainly by NKs) plays a key in processes such as initiation of vascular remodeling of endometrium, angiogenesis in implantation site and even maintenance of the maternal part of placenta. But in spite of this, inhibition of IFN-gamma secondary to inhibition of NKs is the main reasoning of low dose aspirin administration defenders (43). Conversely, IFN-gamma level were lower in women with 20th-25th week pre-term delivery (49). Recently, it has been observed by Sun et al. that blocking Tim-3 is associated with reduction of IFN-gamma and TNF-alpha (27).

In addition to the above data about the role of inflammation, recently a new technique under the title of "endometrial scratching" is proposed (62). Due to novelty of this technique there were a few free-access related articles like Nossair et al (59) and Singh et al (63). In this technique, via making a scratch-suction on endometrium, we inflame endometrium (up to 6 mm thickness) to prepare it for implantation (64). As Nossair et al announced, induction of inflammation can induce decidualization. They believe that although this technique has never been assayed for potential genetic sequels, the obtained embryos were of good-quality.

Since Longman's Embryology counts the teratogenic effect of aspirin and heparin as controversial (52), our search showed that this issue were proposed since 1971 when Kimmel et al (65) had investigated it in high dose and nowadays in 2015 this effect is proved in high doses (66) and probably in low doses like 8 mg/kg in rabbit (67).

Conclusion

As mentioned before, we should not expect more from ARTs. This apparently low success rate for ART is not merely a reason to approach a non-proved therapeutic protocol. If RSA was proved or ARTs do not work after a year of continuous attempt we should seek a remedy; but we should always use a valid protocol to prevent from formation of bad-quality and malformed embryos. We should regard that if the immune system rejects an embryo, it might be due to malformation.

At the end of the review it is notified and suggested that using a valid protocol is necessary for infertility clinical centers. The protocol mainly consists of HLA-C and G typing of the embryo by chorionic villus sampling or amniocentesis and also KIR typing of the both parents with help of the gel-based, serological and genetic techniques.

Ethical Issues

Not applicable.

Conflict of Interests

All the authors declare that there is no conflict of interest.

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