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Implantation and the Fetal Health

Aleksandar Ljubic, Dzihan Abazovic, Dusica Ljubic, Andrea Pirkovic and Andjela Perovic

Abstract

Implantation is one of the crucial periods in human reproduction. Increasing body of evidence suggests that the improper (dysfunctional) implantation and the formation of the placenta can endanger life and health of both the fetus and the mother, during prenatal life and decades after delivery. The idea of the inverted pyramid of prenatal care has emerged in the recent years, as the early detection and prevention of health disorders of the fetus are specially focusing on the first trimester. By applying this principle, disorders in the perinatal period could be prevented or treated with better outcome. The changes that lead to the deficient implantation should be sought in the preimplantation period, in relation between the embryo and the endometrium. It is possible that the time is approaching when the disorders of the pregnancy caused by dysfunctional implantation would be the indication for the application of a natural IVF (without ovarian stimulation) with the use of new biotechnological achievements. For better results of the perinatal medicine, it is necessary to apply earlier (in the preconception and preimplantation periods) the therapy based on the subcellular and genetic level by applying the latest biotechnological procedures.

Keywords: implantation, fetal health, pregnancy complications

1. Introduction

Most of the fetal and maternal complications become apparent with advancing gestation. However, since very important complications that occur later in pregnancy can be predicted in the first trimester, the focus has been set on the evaluations in early pregnancy, thus inverting the pyramid of prenatal care. Although vast majority of early screening tests have been developed and employed, the outcome of pregnancies with the major obstetric syndromes still fails to be significantly improved. The changes etiologically and pathophysiologically associated with disturbed placentation and responsible for the perinatal mortality and morbidity should be sought even earlier, in the preimplantation period, in relation between the embryo and the endometrium.

2. Early pregnancy screening tests and algorithms

The idea of inverted pyramid of prenatal care has emerged for the purpose of prediction and prevention and then early detection and treatment of health disorders of the fetus. By applying this principle, a number of disorders could be prevented or treated with better outcome: fetal aneuploidy and anomalies, miscarriage,

stillbirth, preterm delivery, preterm premature rupture of membranes, preeclampsia, and intrauterine growth restriction [1].

In recent years, screening for aneuploidies during the first trimester has reached effectiveness of over 90% in identifying the most common aneuploidies by a combination of maternal age, fetal nuchal translucency, as well as analysis of free beta-hCG and pregnancy-associated plasma protein A (PAPP-A) [2]. Effectiveness of screening for potential aneuploidies was further augmented with the introduction of the noninvasive prenatal testing using maternal plasma cell-free (cf) DNA, as a secondary test in those patients already regarded as being at high risk. The detection rate of major aneuploidies with this test is up to 99. 3%, with false positive rate of 0. 11% [3].

The development of sonography and MRI diagnostics has led to a growing number of early detected anomalies. A large number of these anomalies can be detected already at 11–14 weeks, while a number can only be found at a later gestation [4]. The prenatal detection rate for the major anomalies is around 68% (varying from 33 to 96%) [5, 6].

First trimester screening often focuses on fetal aneuploidy and major structural anomalies. However, certain maternal characteristics, such as the age and body mass index (BMI), have shown to be very informative, with regard to the predicting miscarriage and stillbirth. The risk of preterm delivery is determined by algorithms that combine these results of the first trimester screening for aneuploidy, increased nuchal translucency, the abnormal ductus venosus flow, and low level of PAPP-A, with the characteristics of the mother [7–9]. Such example is the information on the length of the cervical canal from 11- to 13-week gestation [9, 10]. The risk of spontaneous preterm delivery is associated with cervical shortening in the second trimester, as well as in the first trimester. Combining this parameter with fetal aneuploidy analyses and major structural anomaly results is likely to be used in the future to select a high-risk group that may benefit from close follow-up.

Another example is the screening for the development of early preeclampsia (PE), based on the combination of maternal risk factors, mean arterial pressure, maternal serum PAPP-A, uterine artery Doppler, and placental growth factor. This algorithm has a 95% detection rate for a false-positive rate of 10% [11, 12]. Also, different angiogenesis-related biomarkers; antiangiogenic proteins, like soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin; or proangiogenic proteins, placenta growth factor (PlGF) and vascular endothelial growth factor (VEGF) have been pointed out by a number of authors [10]. The placental protein-13 and other markers, disintegrin and metalloprotease-12 (ADAM12), activin A, or inhibin A, and other microelements or antioxidants, in isolation or in combination, were evaluated in order to predict complications of pregnancy [13–15]. It was also shown that early administration of low-dose aspirin (60–80 mg), starting from the first trimester, reduces the incidence of intrauterine growth restriction as well as its related pregnancy and neonatal complications for 17%, with number needed to treat (NNT) 72, and 14% reduction in fetal or neonatal deaths with NNT 24 [16].

The detection of the small for gestational age (SGA) fetuses could be predicted by algorithms with the combination of maternal characteristics, mean arterial pressure, uterine artery Doppler, and the measurement of various placental products in maternal blood at 11–13 weeks, at a false-positive rate of 10%, about 75% of pregnancies without preeclampsia delivering SGA neonates before 37 weeks and 45% of those delivering at term [17]. Screening for macrosomia by a combination of maternal characteristics and obstetric history with fetal NT and maternal serum-free ß-hCG and PAPP-A at 11–13 weeks could potentially identify, at a false-positive rate of 10%, about 35% of women who deliver macrosomic neonates [18].

Despite the introduction of the vast early pregnancy screening tests, there is still a very slight decrease or even increase in the rate of preterm birth and almost a constant rate of miscarriage, stillbirth, preeclampsia, and SGA [19–23]. Except for hereditary and structural disorders of the fetus, other disorders are etiologically and pathophysiologically associated with disturbed placentation and responsible for the perinatal mortality and morbidity [24].

The reasons why the modern medicine still fails to significantly improve the outcome of pregnancy with the major obstetric syndromes should be sought in the earlier period of pregnancy, even before the conception, and on another subcellular level (Personal communication Dudenhausen, Tirana 2015). Because the consequence of these disturbances is seen in dysfunctional placentation, their sources must be searched before the time of implantation. This means that the changes that lead to the insufficient implantation should be sought in the preimplantation period, in relation between the embryo and the endometrium.

The onset and progression of pregnancy require the coordinated implantation of the embryo and trophoblast invasion into the receptive maternal decidua, followed by proper remodeling of the spiral arteries. Proliferation, migration, and invasion of trophoblastic cells into the maternal endometrium are the essential steps. If any of these steps fails to complete properly because of the endometrial dysfunction, the consequences would be the basis for development of obstetric complications.

3. Maternal health at preconception period

The implanting embryo physically establishes connections with the mother through the endometrium, by a fine-tuned and synchronized crosstalk necessary to support the feto-placental development and health throughout gestation [25]. Early alterations of endometrial physiology can affect the development of the conceptus and the success of pregnancy. The overall status of maternal health is reflecting on the endometrium. If we agree that the optimally prepared mucous membranes (either endogenously, by its own sex hormone or by exogenous regimes) are one of the preconditions for the successful implantation, then the modification of its preparation could influence the occurrence of disorders in later pregnancy and after the birth of the child.

Several studies have shown that preimplantation embryos are sensitive to environmental conditions in which it develops, either in vitro or in vivo, for example, in response to culture conditions or maternal diet [26]. Those conditions can affect future growth and developmental potential, both pre- and postnatally. Recent findings have demonstrated that perturbations of the maternal physiology during the peri-conceptional period (e.g., maternal diet) have impact both on preimplantation phenotype and long-term development and could lead to impaired health during adulthood [26]. Emerging evidence suggests the metabolic status of the mother may "program" the offspring's long-term risk of metabolic disease [27].

Modifications of preimplantation embryo conditions, using assisted reproductive technologies (ARTs) or somatic cell nuclear transfer (SCNT), have been associated with developmental abnormalities and postnatal consequences such as the large offspring syndrome (LOS) in animals [28–30]. Early alterations of the maternal or embryo environment may affect the quality of the embryo-endometrium crosstalk that further leads to pregnancy failure or postnatal detrimental consequences.

4. Endometrium

Before the embryo can implant in the endometrium, the endometrium must be in a receptive state. As a result of a series of timed hormonal events, the so-called window of implantation is opened, which is the time most suitable for the endometrium to support trophoblast-endometrial interaction [31].

The term windows of vulnerability (WOV), i.e., period of time when the endometrium is subject to the influence of factors that may disrupt implantation conditions, has recently been introduced within the framework of reproductive medicine, besides the window of implantation (WOI), i.e., the optimal period of time of activation of endometrial receptivity (De Ziegler, personal communication, MSD symposium, Barcelona 2016).

Prepregnancy approaches such as weight management, blood pressure and blood sugar control, smoking cessation, and optimization of the pregnancy interval may improve implantation and placentation and lead to better pregnancy outcomes [32].

There are a number of different treatment protocols for the "inadequate" endometrium. The medical treatment with estrogens, vasodilators, and sildenafil citrate has neither led to significant improvements of morphological parameters nor to the results in terms of increasing implantation and reduction of the number of miscarriages [33, 34]. There have been reports of trials with immunoglobulins and anticoagulants in pregnancy complication prevention [35–39].

The local endometrium therapy is ongoing for several years. One of the promising therapeutic targets is the corticotropin-releasing hormone (CRH). During implantation, corticotropin-releasing hormone plays a key role in facilitating endometrial decidualization and early maternal tolerance. The embryo implantation provokes the maternal endometrial response similar to the invading semi-allograft that produces acute inflammatory response. After the implantation, the embryo suppresses this response and prevents the rejection [40]. The deregulation of expression pattern of CRH was associated with unfavorable reproductive outcomes as well as chronic endometrium-derived inflammatory disorders, such as endometriosis and adenomyosis [41]. Positive outcome was found after the intrauterine administration of autologous peripheral blood mononuclear cells (PBMCs) [41] especially when pretreated with corticotropin-releasing hormone that acts by regulating apoptosis of activated T- lymphocytes at the implantation site [42]. The results of eight studies showed that intrauterine administration of activated autologous peripheral blood mononuclear cells prior to embryo transfer improves the reproductive outcomes in women with repeated implantation failure [43].

Besides endometrial receptivity, another very important parameter is the endometrial thickness. Defined minimal thickness at approximately 7 mm and clinical pregnancy rates after embryo transfer increase with increasing endometrial thickness. One of the new therapeutic approaches to improve endometrial thickness is the intrauterine perfusion with granulocyte colony-stimulating factor (G-CSF). In clinical reproduction, G-CSF has been proposed as a treatment for implantation failure and repeated miscarriages, two indications for which a US patent has been issued. These authors have applied the drug subcutaneously [44]. Gleicher's papers on flushing uterus cavity with growth factors before the embryo transfer have proposed granulocyte colony-stimulating factor as the treatment of implantation failure and repeated miscarriages [44]. Chang reported successful endometrial expansion in a small group of women with thin endometrium resistant to standard treatments, who were able to proceed to embryo transfer and conceive after uterine perfusion with G-CSF [45].

The use of platelet-rich plasma (PRP) to improve endometrial receptivity is gaining increasing attention in assisted reproduction technologies. Platelets contain a significant amount of growth factors that have positive effects on local tissue repair and endometrial receptivity. Several authors have reported autologous PRP intrauterine injection improves pregnancy and birth rates, especially in patients presenting poor endometrial growth [46].

Chang and associates have recently published the attempt to improve the quality of endometrial thickness, implantation rate, and pregnancy success and to reduce the complications and miscarriage rate, by flushing the uterus cavity with autologous platelet-rich plasma in preparation for the implantation during IVF process [47]. Farimani reported the first successful pregnancy after administration of PRP in a woman with recurrent implantation failure [48]. Kim et al. suggested that the use of autologous PRP improved not only endometrial thickness but restored the endometrial receptivity of damaged endometrium and increased the implantation, pregnancy, and live birth rates (LBR) of the 24 patients with refractory thin endometrium [49]. This therapy delivers biological growth factors, PDGF, TGF-b, and VEGF, insulin-like growth factor 1, epidermal growth factor (EGF), and epithelial cell growth factor to the endometrium. Our group has, so far, treated 25 patients with PRP technology and has achieved a significant improvement of the implantation rates and in reducing the number of abortions. We reported the first case of human embryo obtained after autologous platelet leukocyte-rich plasma (PLRP) in vitro activation of ovaries by interrupting Hippo signaling and PLRP stimulating AKT pathway with ultrasound-guided orthotropic re-transplantation [50]. The patient was a case of an early menopausal woman for whom the ovarian cortex was frozen, thawed, and treated with autologous PLRP which was then transplanted into her menopausal ovaries. Two months after the procedure, follicle formation was noted, and an egg was retrieved resulting in a single embryo [50].

The human endometrium is a dynamic tissue that undergoes monthly cyclic changes, including proliferation, differentiation, and degeneration. Apoptosis is the common pathway of cell death for eliminating senescent endometrial cells from the functional layer of the human endometrium during the late secretory phases of the cycle. It has recently been implied that autophagy is involved in the endometrial cell cycle affecting apoptosis and is the most prominent during the late secretory phase [51]. It is known that the impact on autophagy processes in the endometrium may lead to a reduced incidence of pregnancy complications related to the implantation. Our group has proved that autophagy, a process of controlled self-digestion involved in cellular homeostasis, is dysregulated in endometrial tissue of polycystic ovary syndrome (PCOS) patients and that treatment with metformin might influence endometrial autophagy in PCOS [52]. Other studies reported that metformin can improve endometrial receptivity, enhance endometrial vascularity and blood flow, and revert endometrial hyperplasia and carcinoma into normal endometria in addition to improving hyperandrogenism and insulin resistance in some women with PCOS [53, 54].

5. Embryo environment

The essential requirements for normal implantation and subsequent placentation leading to a healthy gestation are receptive endometrium and healthy embryo. However, there is still a growing number of unexplained failed implantation outcomes that could not be assigned to known factors and require further investigation.

In recent years it was indicated that different etiologies of infertility arose as a result of the underlying genetic and epigenetic changes that contribute to the endometrial dysfunction and lead to implantation failure, miscarriage, and adverse outcomes. These epigenetic and genetic changes lead to placentation defects and contribute to the short- and long-term outcomes associated with infertility. One of the main causes for altered genetic and epigenetic regulation of embryo development and placentation was assigned to hormonal and nutrition-related changes in maternal environment. Embryos respond to the in vivo maternal environment during gestation or during cultivation in vitro in multiple ways that can influence their future growth and health. Developmental plasticity could be altered by the changes in imprinted gene expression, nutrient, and stress-related signaling pathways or cell cycling and apoptotic rates. Embryo phenotype changes through a complex network of interactions with a central role for maternal-fetal neuroendocrine signaling [55]. Maternal undernutrition during gestation alters maternal steroid hormone levels, including elevation of glucocorticoids (GC; corticosterone, cortisol), the stress hormones, which can alter the physiological condition of the conceptus and affect the intrauterine fetal and postnatal growth and cardiovascular and metabolic physiology and enhance the risk of adult-onset disease. This exposure of the embryo to glucocorticoids can alter the fetal hypothalamus pituitary adrenal (HPA) axis, leading to increased fetal GC activity which can, in turn, modify the expression of many downstream-regulated genes that control growth and metabolism, including cardiovascular and renal physiology [55].

The influence of placental function and placental/fetal exchange on fetal programming has been in focus of the recent research. Now it has become widely accepted that maternal nutrition can have the long-term consequences on the offspring without necessarily affecting the size at birth. Altered embryo phenotypes induced by prenatal nutrition are associated with epigenetic modifications. Many imprinted genes contribute to placental function and nutrient exchange [56]. Early epigenetic effects in embryos caused by environmental conditions can lead to physiological impairment to growth due to reduced nutrient supply. There is now evidence from human studies and animal experiments that show the overnutrition and undernutrition during the prenatal period which have lifelong health effects for the offspring and induce the development of noncommunicable diseases during postnatal life [57].

Besides nutrition, the hormonal milieu at conception is known to affect a number of imprinted genes that are expressed during the preimplantation period. Hormonal status will be especially affected during fertility treatment, mostly during IVF. Because superovulation could lead to altered expression of endometrial genes critical to tissue remodeling and placentation, hyperstimulated hormonal status has been implicated in an increased risk for pregnancy complications related to abnormal placentation [58]. Although global methylation pattern was found to be similar among the IVF and spontaneous conceptions early during placentation in the first trimester, differential methylation has been identified in multiple loci between IVF and non-IVF fertility treatments pregnancies but not when compared with spontaneous conceptions. This suggests that there are differences in the infertile population that might be linked to specific treatments, including the hormonal hyperstimulation, that could affect gene imprinting [59]. Several studies have found that the use of ARTs is linked with irregular DNA methylation in human gamete, embryo, placenta, and umbilical cord samples [60, 61]. There were also studies that showed association between specific procedures with methylation differences in placenta, suggesting that specific fertility treatments affect the placental epigenome and function [62].

6. Embryo monitoring

The embryo has, in addition to the endometrium, the crucial importance for the success and regularity of the implantation and then placentation. The morphological assessment of the embryos' quality is insufficient for the cognition of its biological resources. The new invasive and noninvasive techniques of embryo quality assessment have been developed. Nowadays, the invasive technology means preimplantation genetic testing (PGT), the aneuploidy screening, or diagnosis of specific genetic disorders of the embryo before the transfer by using next-generation sequencing (NGS). These tests include biopsy trophectoderm cells with blastocyst vitrification [63–65]. With trophectoderm biopsy, both maternal and paternal abnormalities can be studied. Possible disadvantages are the presence of mosaicism and the fact that the trophectoderm might not be a representative of the inner cell mass.

Noninvasive time-lapse embryo monitoring allows continuous embryo observation without the need to remove the embryo from optimal culturing conditions. The information on the cleavage pattern, morphologic changes, and embryo development dynamics could help us identify embryos with a higher implantation potential. It has also been shown that imaging phenotypes reflect the molecular program of the embryo, where individual blastomeres develop autonomously toward embryo genomic activation [66].

This type of monitoring allows for the collection of much more information on the timing of the cleavages and the dynamics of the morphologic changes, with analysis of the kinetics of the events up until the blastocyst stage [67].

Various kinetic and morphologic markers have already been found that are associated with the minimal likelihood of implantation and others that are predictive of blastocyst development, implantation potential, genetic health, and pregnancy [68, 69].

7. Gametes

After the formation of the embryo, its fate is already determined. The gamete quality has the crucial part in the creation of the high-quality embryos. The conditions, in which oogenesis and spermatogenesis take place, have a crucial impact on the quality of embryos that is formed from these gametes.

7.1 Oogenesis

The evaluation of the oocyte quality based on morphological evaluation is not sufficient for an insight into the biological potential. It can identify those cells that have nuclear immaturity, significant degeneration, or major abnormalities. Recently, the developed strategies including the genomic, transcriptomic, and proteomic approaches have been applied in assisted reproduction. Their goal is to identify a "molecular profile" of embryo development by detecting the chemical components in the oocyte, granulosa cells, follicular fluid, and embryo culture medium [70].

Better predictors, the birefringence properties of the meiotic spindle, and the zona pellucida are indicative of good health of the oocyte [71]. A very useful data can be obtained from the application of studying gene expression from cumulus cells, using microarrays, as biomarkers for oocyte viability. The metabolomic profiling of oocyte spent culture media by mass spectroscopy has shown differences related to oocyte maturation, embryo development, and implantation

success [72]. Oocyte quality can be assessed by the measurement of oocyte oxygen consumption [73].

Spermatogenesis: the quality of spermatogenesis is the condition for the formation of a good embryo. The advanced sperm selection techniques are based not only on the morphological assessment (defragmentation, MACS) but also on the evaluation of specific cellular characteristics (membrane integrity, density, surface charge) that provide a choice of better quality sperm. The methods of improving conditions of gametogenesis, which are applied so far, do not provide a sufficient effect. They are mainly related to the balance correction of microelements and vitamins, as well as the oxydo-reductive processes in the body. The sperm chromatin and DNA integrity are necessary to ensure normal embryo development. It is now clear that DNA damage in spermatozoa has a negative influence on blastocyst development and the pregnancy outcome [74] . Similarly, centrosome integrity is critical for successful fertilization and embryo development. There are studies that have described the association between sperm with DNA damage and a history of recurrent miscarriage [75].

7.2 Advanced therapy

Magnetic-activated cell sorting (MACS) technology for sperm could improve obstetric and perinatal outcomes compared with those achieved after swim up. Treatment of sperm with MACS procedure prior to IVF results in a marked improvement in pregnancy rate and cessation of the abortion rate in couples whose ejaculates initially had high levels of SDF [76].

A number of prerequisites are needed to create high-quality oocytes, those conditions are likely to be grouped into several parts: the existence of high quality responsive oogonia, its potential of the adequate number increase and quality of mitochondria, the presence of sufficient amounts and types of growth factors, orchestrated by the balance of blocking (Hippo) and activating (ACT) gene pathways [77].

For decades it was believed that the woman's reproductive potential is entirely dependent on the size of the stock (pool) of primordial follicles in the ovary. The paradigm that has prevailed for decades in the scientific world about the existence of a consistent number of primordial follicles, established during embryonic and fetal period, was in many ways changed by Tilly's group work. They practically demonstrated the existence of germline or oogonial stem cells [78].

Their dormant status is characterized by communication with surrounding granulosa cells and numerous mechanical and chemical factors controlling the progression of their cell cycle. These factors control signaling activation of the pathways included in the primordial follicle dormant status regulation, like Hippo and AKT signaling [77]. During the recent years, various programs have been developed to try to improve the quality of oocytes. It has been shown that it can be influenced on the activation of primordial cells and maturation to the mature oocyte. The stem cells can be influenced by the stem cell therapy in order to obtain the intracellular communication with the existing ovarian primordial oogonia. The therapy with mesenchymal stem cells has led to the recovery features of oocytes after the chemotherapy-induced insufficiency [79]. The animal experiments by the in vitro therapy with developed stem cells have led to the birth of live offspring without abnormalities [80]. Other groups of authors have tried to improve the ovarian function with the growth factors obtained from the plasma and enriched with platelets and leukocytes. The cases of childbirth after re-transplantation of ovaries with support of PRP have been published [81]. Our group has achieved a normal pregnancy outcome after the sonographically guided therapy with growth factors in a female patient aged 40 years, after 18 attempts of in vitro fertilization.

The role of the number and function of mitochondria in the development of quality oocytes is surely very important. The problems of mitochondrial heteroplasmy go with the complicated, technologically very complex methods of polar body transfer, spindle transfer, and pronuclear or oocyte transfer [82–84]. The augmentation of autologous mitochondria carries a potential treatment. Our team has inaugurated the attempt of the mitochondrial energy boosting with ovarian high-intensity interval training (HIIT).

The autologous growth factors that are intraovarian instilled are leading to the changes in the production and efficiency of the local growth factors. The influence on the genetic control of oogenesis, by the modification of the Hippo and AKT signaling pathways, is possible in different ways. The correction of the gene signaling or autologous tissue genetic bioengineering is certainly a step forward in obtaining the quality gametes [50, 84].

8. Conclusion

Implantation is one of the crucial periods in human reproduction. Increasing body of evidence suggests that the improper (dysfunctional) implantation and the formation of the placenta can endanger life and health of both the fetus and the mother, during prenatal life and decades after delivery. The changes that lead to the insufficient implantation should be sought in the preimplantation period, in relation between the embryo and the endometrium. It is possible that the time is approaching when the disorders of the pregnancy caused by dysfunctional implantation would be the indication for the application of a natural IVF (without ovarian stimulation) with the use of new biotechnological achievements. For better results of the perinatal medicine, it is necessary to apply earlier (in the preconception and preimplantation periods) the therapy based on the subcellular and genetic level by applying the latest biotechnological procedures.

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