REVIEW ARTICLE

Ultrasound and Biologic Therapy in Reproductive and Perinatal Medicine

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Abstract

New biotechnology procedures have been introduced to the field of reproductive and perinatal medicine to find solutions for the successful reproduction, as well as disturbances responsible for maternal and perinatal morbidity. Ultrasound plays a crucial role in the early detection of reproductive disorders and perinatal medicine, where biological therapies can be possibly applied. Besides, these techniques require precise monitoring of the application of the biological agent, for which ultrasound is a sovereign method. The use of autologous sources, such as platelet-rich plasma and bone marrow-derived stem cells, provides a wide range of therapeutic strategies for gonadal failure and endometrium therapy. Stem cells from amniotic fluid could be used as the sources for direct fetal treatment in different fetal disorders (neurological disorders, the intrauterine growth restriction). Maternal complications, such as premature rupture of the amniotic sac and disturbed placental adherence, are being successfully treated with the use of biological autologous fibrin tissue adhesives. Postpartum complications related to the change of the pelvic floor could be prevented by the peripartal local application of growth factors and/or stem cells. Subcellular therapies, as microvesicles/exosomes are membrane-bound biological nanoparticles secreted from stem cells. These particles are variable source that could act as another source of factors that could affect molecular cascades, emerging as a new diagnostic and therapeutic tool in reproductive and perinatal medicine.

Keywords: Biotechnology, Exosomes, Platelet-rich plasma, Stem cells, Ultrasound.

Donald School Journal of Ultrasound in Obstetrics and Gynecology (2020): 10.5005/jp-journals-10009-1647

Introduction

The introduction of the biotechnological procedures into the perinatal and reproductive medicine aims at primarily improving the implantation and the problems related to it by decreasing the number of complications in both early and late gestation. Moreover, biological therapies are increasingly used in the treatment of the already emerged complications, such as premature rupture of membranes (PROM).

The complications that occur during pregnancy may result from stress and psychosocial issues (including social support, anxiety, and other psychological variables). They may involve the mother's health, the health of the baby or both. Several women have health issues before pregnancy, which can cause complications. Other problems appear during the period of pregnancy. The complications may be common or rare, but there are treatments and therapies which may resolve all the problems that appear during pregnancy.

The main goals of the prenatal ultrasound examination performed between the 11th and 14th week of pregnancy are to establish the exact gestation point, identify multiple pregnancies, and determine amnionicity and chronicity, as well as to screen the significant fetal anomalies and structural disorders.[1] Certain complications that may occur late in pregnancy can be predicted during the first trimester. Some anomalies may be detected even in the period between the 11th and 14th week using algorithms that combine the following: Sonography results, levels of specific biochemical parameters, and the characteristics of the mother. The aforementioned combined algorithm estimates the risk of the most common chromosome aberrations, as well as other possible complications related to the pregnancy itself. An early risk assessment of potential pregnancy complications could improve the outcome by a different organization of the prenatal care, which would be based on an individual approach to a patient and disorder, rather than on a series of routine visits.[2] The screening for aneuploidy during the first trimester has lately become efficient for over 90% in identifying the most common aneuploidies by combining the mother's age, measured nuchal translucency, and levels of the free beta-human chorionic gonadotropin (hCG) and pregnancy-associated plasma protein A (PAPP).[3] The screening efficiency on potential aneuploidies is additionally increased by the introduction of the noninvasive prenatal examination using

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How to cite this article: Ljubić A, Abazović D, Draganić VD, *et al.* Ultrasound and Biologic Therapy in Reproductive and Perinatal Medicine. Donald School J Ultrasound Obstet Gynecol 2020;14(3): 214–219.

Source of support: Nil

Conflict of interest: None

the free fetal DNA in the plasma (cf), as a secondary test for those patients who are already regarded as being exposed to high risk. The detection ratio of the main aneuploidies reaches 99.3% using this test, with the figure of false-positives being 0.11%.[4]

The development of sonography and magnetic resonance (MR) diagnostics has considerably contributed to the early detection of anomalies. A large number of these anomalies may be detected early, during the period between the 11th and 14th week, whereas a few may be found only in late pregnancy.[5] The prenatal ratio of the detection of major anomalies is around 68% (varying from 33 to 96%).[6,7]

The first-trimester screening is mainly focused on fetal aneuploidies and large structural anomalies. However, specific characteristics of the mother, such as their age and body mass index (BMI), have proven quite helpful in predicting adverse outcomes, such as miscarriage and stillbirth. The risk of a premature delivery can be assessed using the algorithm that combines the screening results of the first trimester, which presuppose a larger thickness of the nuchal translucency, an abnormal flow in the ductus venosus, and a low level of PAPP, with the characteristics of the mother.[8–10] Cervicometry illustrates one of the variables which may predict the risk of premature birth in the period between the 11th and 13th week of gestation.[11] The risk of a premature miscarriage is closely connected with the shortening of the cervix length in the first and second trimester. The combination of this parameter with the previous screening analysis of the first trimester is likely to be used for determining high-risk patients. They may benefit from intensive monitoring and observation.

Another example is the screening for preeclampsia (PE), based on the combination of the risk factors of the mother, arterial pressure, the mother's serum values PAPP, uterine artery Doppler, and the growth factors of the placenta. This algorithm has the detection ratio of 95%, while the percentage of false-positives is 10%.[12,13] Numerous authors have emphasized various biomarkers related to the angiogenesis: Antiangiogenic proteins, such as the soluble fms-like tyrosine kinase 1 (sFlt-1), soluble endoglin or proangiogenic growth factor of the placenta (PlGF), and vascular endothelial growth factor (VEGF). Placenta protein-13 and other markers disintegrin and metalloproteinase-12 (ADAM12), active A or inhibin A, as well as other microelements or antioxidants, either isolated or in combination, may be used for predicting certain pregnancy complications.[14–16] An early treatment with aspirin in low doses (60–80 mg) starting from the first trimester lowers the risk of the intrauterine growth restriction.[17]

Fetal morbidity and mortality of the mother during pregnancy represent important issues of public health.[18] Fetal mortality refers to the intrauterine death of the fetus in any period of gestation. According to the national research conducted in the USA, about one million fetal deaths occur annually, the majority of them happening before the 20th week of gestation.[19] The data on the fetal mortality offered by the National Vital Statistics System relates to the period after the 20th week of pregnancy. Even if only the deaths of fetuses aged 20 or more weeks are considered, the rate of fetal mortality is almost identical to the rate of child mortality in the USA.[20] Perinatal mortality refers to the death immediately prior to delivery and includes fetal mortality (at least 20 weeks of pregnancy) and neonatal mortality.

The majority of complications in fetuses and mothers become evident with the progression of gestation. Regarding the fact that some very important complications likely to appear in late pregnancy can be predicted in the first trimester, the focus of attention is placed on the evaluation in early pregnancy, which inverts the pyramid of the prenatal care.

Since the previous attitude to the perinatal medicine is only partially successful in decreasing the number of the most important pregnancy complications (including the congenital anomalies and multiple pregnancies), it is necessary to develop a different approach, which is presented by the aforementioned inverted pyramid.

The idea of the inverted pyramid of the prenatal care has emerged to predict and prevent, and eventually early detecting and curing the fetal health disorders. The application of this principle may prevent numerous disorders. Additionally, it may treat them with a better outcome, such as fetal aneuploidies and anomalies, miscarriage, stillbirth, premature delivery, preterm PROM, PE, and intrauterine growth restriction.[21]

Era of Biological Therapies

The reasons why modern medicine is still unable to improve the outcomes of pregnancies accompanied with serious complications considerably are to be found in the period of early pregnancy, even before the conception, as well as at the subcellular level. Since the consequence of these disorders is noticeable in the unfunctional placentation, their sources must be searched for in the time before implantation. Therefore, the changes that cause inadequate placentation are to be found in the preimplantation period, in the relationship between the embryo and endometrium.

The beginning and progression of pregnancy require coordinated implantation of the embryo with the trophoblast invasion into the receptive decidua of the mother. The fundamental steps include proliferation, migration, and invasion of the trophoblast cells in the endometrium. The obstetric complications will be developed if those steps are not performed appropriately due to the dysfunction of the endometrium.

Stem cells are defined as the cells from which all other cells in the human body are developed, i.e., the autologous, allogeneic, or xenogeneic cells that are multiplied, expanded, selected, treated pharmacologically, as well as their products or otherwise altered in their biological characteristics *ex vivo*, so that they may be applied to humans and used in the prevention, treatment, diagnostics, or mitigation of diseases or injuries.[22] A tightly linked network of physiological, cellular, and molecular paths supports the development of a healthy fetus. The goal of new biotechnological procedures is to reach the solutions for the disorders responsible for maternal and perinatal mortality. There is a connection between numerous mutually dependent tissues of the mother and the embryo. The restoration of an appropriate signalization through targeted biological therapies may result in an improvement of the fetus and a better pregnancy outcome.

As regards those mentioned above, the application of the biological preimplementation therapy should be emphasized as contributing to a considerable improvement of the rate of accomplished pregnancies by operating on a high-quality cell, seed, and implementation process.

Endometrium

The endometrium has to be in the receptive state before the embryo can be implemented into the endometrium. A series of successive hormone events results in the so-called "implantation window" which opens up, marking the time which is the most appropriate for the endometrium to support the trophoblast interaction.[23]

In the field of reproductive medicine, besides the window of implantation (WOI), there has been recently introduced the window of vulnerability (WOV), i.e., the time scope in which the endometrium is susceptible to the factors that may impair the implantation conditions or the optimal time of the activation of the endometrium receptivity.[24]

Researchers have long endeavored to define the optimal endometrium into which a quality embryo is transferred. The availability of ultrasonography has enabled an intensive assessment of the endometrium thickness and its characteristics. Researches have determined that the hypoechogenic endometrium is more receptive than the iso- or hyperechogenic endometrium.[25] However, the thickness of the endometrium has remained the point of debate. Although the majority of clinical medical practitioners empirically prefer the endometrium of >7 mm, the available evidence does not support any specific thickness because the pregnancies with similar outcomes are described with the endometrium thickness from 5 to >15 mm.[26]

Monitoring performed prior to pregnancy, which encompasses the control of weight, blood pressure, and sugar levels, giving up smoking and pregnancy interval optimization, may improve the implantation and placentation, and consequently result in better pregnancy outcomes.[27] There are numerous different protocols used for the treatment of an "inadequate" endometrium. The medical treatment with estrogen, vasodilators, or sildenafil citrate drugs has neither improved the morphological parameters significantly nor led to a higher rate of implantation and lower number of miscarriages.[28,29] Studies of the application of immunoglobulin and anticoagulants in the prevention of pregnancy complications have also been reported.[30–33]

Contemporary researches have proven that molecular technologies contribute to a better understanding of the endometrial receptivity.[34] Early changes of the endometrium physiology can have an impact on fetal development and pregnancy. The mother's physiology during the period of conception (e.g., the mother's diet) determines the phenotype of the preimplantation period. One of the factors crucial for a successful implantation is an optimally prepared endometrium (either endogenously, with one's own sex hormones, or exogenously).

The use of the patient's cells and tissue for the preparation of the endometrium may yield the greatest success. It is safe since it will not cause a hypersensitive reaction or transfer of infectious agents.

Our experience has so far confirmed that it is the biological therapy that can considerably solve the issues related to the implantation.

One of the promising therapies is the one with the corticotropin-releasing hormone (CRH). During the implantation, CRH is essential in facilitating the endometrium decidualization and early maternal tolerance. The implantation of the embryo triggers the endometrial response similar to the invasive semiallograft, which causes an acute inflammatory response. After the implantation, the embryo suppresses this response and prevents rejection.[35] The dysregulation of the CRH expression pattern is connected with inauspicious reproductive outcomes, as well as with the chronic inflammation disorders originating in the endometrium, such as endometriosis and adenomyosis.[36] The positive outcome is achieved after the intrauterine application of the autologous peripheral blood mononuclear cells (PBMCs),[37] especially after the previous treatment with human corticotropin-releasing hormone (hCRH), which regulates the apoptosis of the activated T-lymphocytes in the place of implantation. The results obtained in eight studies have demonstrated that the intrauterine application of the activated autologous PBMCs before the embryo transfer improves the reproductive results in the women with repeated unsuccessful implantations.[38]

The thickness of the endometrium is another important parameter, besides the endometrial receptivity. The minimal thickness is defined at 7 mm, while the rate of clinical pregnancies after the embryo transfer increases with the increase of the endometrium thickness. One of the modern therapies for the improvement of the endometrium thickness is the intrauterine perfusion with granulocyte colony-stimulating factor (G-CSF). In medical practice, G-CSF is suggested as the treatment for unsuccessful implantation and repeated miscarriages, two indications for which the American patent has been approved. These authors applied for this medicine subcutaneously.[39] For instance, Gleicher, in his papers on flushing the uterine cavity with the growth factors before the embryo transfer, suggests the G-CSF for treating unsuccessful implantation and repeated miscarriages. Gleicher reports on a favorable increase of the endometrium thickness in a small group of women with a thin endometrium resistant to standard treatments. In such patients, it was possible to perform the embryo transfer, nor induce pregnancy after the perfusion of the uterus with the G-CSF.[40]

The endometrium is a dynamic tissue, subjected to monthly cyclical changes, including proliferation, differentiation, and degeneration. Apoptosis represents a common death path which eliminates the cells during the late secretion phases of the period. Autophagia has been lately considered to be included in the cell cycle of the endometrium that impacts apoptosis and to be the most striking during the late secretion phase.[41] It is known that the impact on the autophagic processes in the endometrium may lead to a decrease in the pregnancy complications related to the implantation. Our group of authors has proven that autophagia, the process of the controlled self-digestion that participates in the cell homeostasis, is dysregulated in the endometrial tissue of the patients suffering from the polycystic ovary syndrome (PCOS), and that the metformin treatment can affect the endometrial autophagia in PCOS.[42] The results of some other studies show that metformin can improve the endometrial receptivity, increase the vascularization and blood flow, and thus restore hyperplasia and endometrium cancer to the normal endometrium, besides improving hyperandrogenism and insulin resistance in some women with PCOS.[43,44]

Plasma Rich in Thrombocytes

Assisted reproductive technologies have become more concerned with the use of the plasma rich in thrombocytes (PRP). Thrombocytes contain a high concentration of the growth factor in the alpha granules. These growth factors include the following: The growth factor originating from the thrombocytes, insulin similar to the growth factor, VEGF, and all other factors that stimulate wound healing together with fibronectin and vitronectin. PRP is used in other medical therapies for tissue regeneration.[45] The autologous PRP is isolated from the sample of the patient's blood, from which red blood cells are excluded centrifugally. The remaining plasma has 5 to 10 times higher concentration of the growth factor than blood. The authors have shown that PRP is beneficial for a bad endometrium by accelerating regeneration and lowering fibrosis. The methods applied include flushing of the endometrium and

subendometrial injection.[46] Several authors have reported that the autologous PRP injection improves the ratio of pregnancy and natality, especially in patients with weak endometrial growth.[47]

Chang et al. have recently published their findings concerning the attempt to improve the quality and thickness of the endometrium, implantation rate, successful pregnancy ratio, and decrease of the complications and miscarriage rate by flushing the uterine cavity with the autologous PRP in preparation for the implantation during the *in vitro* fertilization (IVF) process.[48] Farimani reports on the first successful pregnancy after the application of PRP in women with multiple unsuccessful implantations.[49] Kim et al. suggest that the use of the autologous PRP not only improves the thickness of the endometrium but also increases the implantation, pregnancy rate, and the rate of successful deliveries in 24 patients with a thin endometrium.[50]

This therapy involves the biological growth factors, platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-b), VEGF, growth factor similar to insulin, epidermal growth factor (EGF), and the growth factor of the epithelial cells. Our group of authors published the first case of the human embryo obtained after the autologous platelet leukocyte rich plasma (PLRP) *in vitro* activation of the ovaries by discontinuing Hippo signalization and PLRP stimulating protein kinase B (AKT) pathways, using the orthotropic retransplantation performed by ultrasound.[51] The case involves a woman in early menopause whose ovarian cortex was frozen, unthawed, treated with the autologous plasma rich in thrombocytes and leukocytes (PLRP), and later transplanted into her ovaries in the menopause. The creation of follicles was detected 2 months after the operation. Obtained ovarian cell evolved into an embryo.

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are human cells from fat tissue, bone marrow, peripheral blood, umbilical cord blood, placenta, period blood, mother's milk, or urine. When the tissue is injured, the MSCs produce large amounts of growth factor that regenerate the injured tissue. They are popularly termed "the medical signal cells".[52] Unfortunately, the human organism loses its regenerative ability with age. People lose an enormous number of their stem cells as they grow old, while those that remain are far less efficient and powerful than the ones people have in their youth. Regenerative medicine is focused on two separate yet complementary goals. First, it is the stimulation of one's stem cells with the purpose of increasing their number and their ability to function. Second, it is the insertion of the stem cells from other sources, so that they can function instead of one's own less capable stem cells. Mesenchymal stem cells are acceptable due to their immune privilege that enables the avoidance of the host's immunological response. Mesenchymal stem cells are completely safe regarding our immune system because they do not provoke an immunological reaction (rejection) from the patient who receives them.

The autologous MSCs from the bone marrow have proven considerably helpful in treating the ovaries damaged by chemotherapy, as well as the ovarian issues related to aging.

The first results obtained from the researchers conducted with human beings demonstrate that the MSC infusion from the bone marrow into the ovaries may promote local angiogenesis, lower apoptosis, and increase cell proliferation in the primordial and antral follicles. Another potential explanation of the effect that MSCs have on the ovarian function is that the MSC signal molecules induce regressive stem cells in the ovary using the paracrine communication.[53]

Biological autologous fibrin tissue fibers can be used for treating the PROM, as well as for preventing blood loss due to the partial placental abruption. The complications related to the changes in the pelvic floor due to the childbirth (vaginal and uterine prolapse, urinary incontinence) can be prevented by a peripheral local application of the growth factor and/or stem cells.[54] Premature rupture of the membranes is caused by the damage in the fetal membranes, which further leads to the development of congenital infections and unfavorable neonatal outcomes. PRP is an *in vitro* model for the estimation of the ability of the plasma rich in thrombocytes to heal iatrogenic wounds created on the fetal membrane which has been tested on a single layer and bilateral amnion models. The experiments have confirmed that the PRP "patch" lasts for almost 2 months in the amnion liquid. It is also a waterproof cure for the iatrogenic damages of amnions and chorions. Moreover, PRP stimulates cell growth and proliferation, improving thus the response to the healing of the membrane.[55]

In Utero Hematopoietic Stem Cell Transplantation (IUHCT)

The first general *in utero* stem cell therapy is most likely to be applied for the treatment of hematological and immunodeficiency diseases using the hematopoietic stem cells (HSCs). Hematopoietic stem cells are capable of self-replication and maintaining hematopoiesis throughout an individual's life even if applied prenatally. In the early phase of gestation, the immune system is in the process of self-education. The immunological immaturity of the embryo enables the import of antigens (i.e., the donor's HSC) without an immunosuppressive response, which results in the induction of the immune tolerance specific for the antigen.[56] This method is effective in treating numerous congenital hematological, genetic, and immunological disorders which can be diagnosed before the childbirth and before the maturation of the fetal immune system owing to the advancement in the field of prenatal care.

The isolation of stem cells from the amnion liquid enables the development of direct therapy for the treatment of various fetal disorders (neurological disorders, intrauterine growth restriction). The amnion liquid consists of the cells of the fetal origin, such as amnion, skin, and respiratory tract, and can be collected during a routine amniocentesis, which is a minimally invasive procedure used for the prenatal diagnostics.[57] Its advantage is the possibility to avoid the rejection of the transplant. The autologous stem cells, isolated from the amnion liquid and characterized, may be further modulated *in vitro* and then retransplanted in some other place.[58] The development of biological subcell therapies additionally increases the possibility of their application in the fetal period.

Exosomes

Exosomes are the so-called extracellular vesicles, or bubbles, released from cells, the stem cells in particular. They function as shuttles transporting certain genetic information, proteins, and RNA for other cells. This commonly occurs as a response to injuries. In general, exosomes carry healthy and lost information which they insert into the targeted cells. They enable the cell-to-cell communication, transfer the molecules which regulate important intracellular information between close and distant cells. They carry information from one spot to another and give instructions to cells how and when to react. The exosomes from the stem cells contain

precious cellular information — proteins and genetic information, essential for the appropriate functioning of the cell. The exosomes obtained from the stem cells assist the transformation of the natural killer cells (NK cells) into the regulatory T cells (helper T cells). They aid the balancing of an overactive immune system and modulate it in such a way that it reacts in a coordinated manner. Besides, they may be used as the system for the delivery of medicines. Exosomes impose themselves as the next generation of cell therapy. Although they are not cells in the true sense of the word, exosomes play a vital role in the communication and rejuvenation of all the cells in the human body. It has been proven scientifically that cell-to-cell communication is essential for the maintenance of healthy cellular terrain. Old age, chronic diseases, environmental factors, and genetic disorders can hinder how human stem cells communicate with other cells and thus derange the healing process.

Exosomes are of crucial importance for the regulation of these communication processes. This research is a significant insight into the practical functionality of exosomes. The exposure of the cells of an old human organism to those of the young one proves that the exosomes from immature stem cells are crucial for the rejuvenation of the old cells.[59] This healing mechanism can be used in regenerative medicine. Exosomes are used for reinforcing the previously performed rejuvenation procedure (SEGOVA). A degenerative disease causes continuous cellular degradation, affecting tissues or organs. While the stem cells are generally responsible for the cellular rejuvenation, external factors can impede and hamper this function. Therefore, they might not be able to offer all the necessary information. The support of their function by external exosomes could exert a positive effect by providing new information that would encourage the healing process.

"Bacterial Baptism"

The epidemiological studies related to the cesarean section indicate a higher rate of certain conditions, such as asthma, allergies, autoimmune disorders, and obesity. An inadequate establishment of the microbiome in young age results in unfavorable outcomes late in life, observed in the babies born by cesarean. These differences may be ascribed to the "bacterial baptism" of the vaginal birth, which is avoided by the cesarean section. The newborns' gastrointestinal tract is believed to be sterile up to its first exposure to microorganisms at the moment of birth when the baby passes through the birth canal and is covered with the microorganisms of the mother or when the baby swallows some of the microorganisms from the mother's vagina.[60] The practice of the "vaginal seeding" is an iatrogenic transfer of the vaginal microbiome to the newly born baby so as to promote the establishment of a "normal" microbiome for the newborns.[61]

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