REVIEW ARTICLE

Implantation and the Fetal Health

Aleksandar Ljubić[1], Dušica Ljubić[2], Tatjana Božanović[3]

Abstract

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. 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.

Gametes: The birefringence properties of the meiotic spindle and the zona pellucida are indicative of good health of the oocyte. 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. Oocyte quality can be assessed by the measurement of oocyte oxygen consumption. The role of the number and function of mitochondria in the development of quality oocytes is surely very important. The correction of the gene signaling, or autologous tissue genetic bioengineering is certainly a step forward in obtaining the quality gametes. The stem cells can be influenced by the stem cell therapy in order to obtain the intracellular communication with existing ovarian primordial oogonia. The sperm chromatin and DNA integrity are necessary to ensure normal embryo development. Magnetic-activated cell sorting technology for sperm could improve obstetric and perinatal outcomes.

Embryo: 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). Noninvasive time-lapse embryo monitoring allows continuous embryo observation without the need to remove the embryo from optimal culturing conditions. 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.

Endometrium: The medical treatment with estrogens, vasodilators, sildenafil citrate has neither led to significant improvements of morphological parameters nor to the results in terms of increasing of implantation and reduction of the number of miscarriages. There have been reports of trials with immunoglobulins and anticoagulants in pregnancy complication prevention, as well as the intrauterine administration of autologous peripheral blood mononuclear cells (PBMCs), especially when pretreated with corticotropin-releasing hormone (CRH) that acts by regulating apoptosis of activated T-lymphocytes at the implantation site. The quality of endometrial thickness, implantation rate and pregnancy success, and the reduction of the complications, miscarriage rate, is attempted by flushing uterus cavity with autologous platelet-rich plasma (PRP) in preparation for the implantation during IVF process.

Keywords: Dysfunctional implantation, Embryo, Endometrium, Gametes, Oocyte, Semen.

Donald School Journal of Ultrasound in Obstetrics and Gynecology (2021): 10.5005/jp-journals-10009-1684

Introduction

Implantation is one of the crucial periods in human reproduction, vital to the maintenance of mankind. At the moment that blastocyst makes contact with epithelial surface of the uterus, placentation is initiated, leading to a series of events known as implantation. At the end of this process, the specialized organ is formed—the placenta, resulting from the interaction of two genetically different individuals—the fetus and the mother. 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.

In the recent years, the prediction and prevention, early detection and treatment of health disorders of the fetus, is focusing on the first trimester. The idea of the inverted pyramid of prenatal care and monitoring has emerged and outlined, for the purpose of prediction and prevention, and then early detection and treatment of health disorders of the fetus. Nicolaides brings forward the disorders in the perinatal period, which, by applying this principle, 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 retardation (IUGR).[1]

[1]Department of Obstetrics and Gynecology, Medigroup Serbia, Belgrade, Serbia

[2]Department of Assisted Reproductive Technologies, Medigroup Serbia, Belgrade, Serbia

[3]Clinic for Obstetrics and Gynecology, Clinical Centre of Serbia, Medical School, University of Belgrade, Serbia

Corresponding Author: Aleksandar Ljubić, Department of Obstetrics and Gynecology, Medigroup Serbia, Belgrade, Serbia, Phone: +381647019632, e-mail: gakljubic@gmail.com

How to cite this article: Ljubić A, Ljubić D, Božanović T. Implantation and the Fetal Health. Donald School J Ultrasound Obstet Gynecol 2021;15(1):81–86.

Source of support: Nil

Conflict of interest: None

Nowadays, it is known that over 90% of most common aneuploidies can be identified by a combination of maternal age, fetal nuchal translucency, as well as analysis of free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A).[2] If we add to this, 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 situation has further changed. The detection rate of major aneuploidies is up to 99.3%, with false-positive rate of 0.11%.[3]

As for major fetal abnormalities, the development of sonography and MRI diagnostics has led to a growing number of antenatal detected anomalies and to the improvement in the treatment outcome. A large number of these anomalies can be detected already during the medical examination from 11 weeks to 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% (vary from 33% to 96%).[5,6]

The connection with certain maternal characteristics, such as the age and body mass index (BMI), is important, with regard to the possibility to predict the miscarriage and stillbirth. The existence of previous miscarriages or stillbirth is also important, as well as, the pathological results of the first trimester screening for aneuploidy, increased nuchal translucency, the abnormal ductus venosus flow and low level of PAPP-A.[7,8] Early detection of risk could lead, by more intensive monitoring of fetal growth and birth and planned delivery, to a reduction in the number of these complications.

The risk of preterm delivery is determined by algorithms that combine the characteristics of the mother and obstetric anamnesis, with information on the length of the cervical canal from 11 weeks to 13 weeks gestation.[9] Predictive accuracy of changes in transvaginal sonographic cervical length over time for preterm birth was also analyzed.[10] The screening for the development of early preeclampsia is based on the combination of maternal risk factors, mean arterial pressure, maternal serum PAPP-A, uterine artery Doppler, and placental growth factor (PlGF). This algorithm has a 95% detection rate for a false-positive rate of 10%.[11,12]

The other authors have pointed to a different angiogenesis-related biomarkers, antiangiogenic proteins, like soluble fms-like tyrosine kinase 1 (sFlt1), soluble endoglin, or proangiogenic proteins PlGF, vascular endothelial growth factor (VEGF).[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, in order to predict complications of pregnancy, were tested.[13–15] Test accuracies of all markers, however, are too poor for accurate prediction of preeclampsia in clinical practice. Low-dose aspirin (60–80 mg), starting from the first trimester improve placentation and reduce the prevalence of the serious disease for 17%, with number needed to treat (NNT) 72, and 14% reduction in fetal or neonatal deaths with NNT 243.[16]

It is also stated that small for gestational age (SGA) fetuses may be predicted by algorithms which combine 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 β-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]

Is this enough? Did we do enough in terms of reducing miscarriages and stillbirths, conception, and development of children with hereditary and/or structural abnormalities, as well as, pregnancy complications, which leads to the greatest number of deaths and morbidity—preterm delivery, preeclampsia, fetal growth retardation? There is abundant data on very slight decrease or even increase in the rate of preterm birth and almost constant rate of miscarriage, stillbirth, preeclampsia, and SGA.[19–23]

Can we do more?[24] Except for hereditary and structural disorders of the fetus, other disorders are etiologically and pathophysiologically associated with disturbed placentation.

If we analyze the period behind us, the progress has been made in the field of detection of multiple fertility, dating of pregnancy and prenatal detection of chromosomal and structural disorders of the fetal structure. There is also a small progress in terms of prevention of preeclampsia. What is the reason for the failure of modern medicine to significantly improve the outcome of pregnancy with the major obstetric syndromes, responsible for the perinatal mortality and morbidity? We believe that the roots of these disorders must be sought in the earlier period of pregnancy (even before the conception), and on the other, deeper, subcellular level (personal communication Dudenhausen, Tirana 2015). If these disorders are the consequence of the disturbed or dysfunctional placentation, then their roots go up to 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.

Preconception Period

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) is 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.

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.[25]

Endometrium

There are a number of different treatment protocols for the "inadequate" endometrium. The medical treatment with estrogens, vasodilators, sildenafil citrate has neither led to significant improvements of morphological parameters nor to the results in terms of increasing of implantation and reduction of the number of miscarriages.[26,27] There have been reports of trials with immunoglobulins and anticoagulants in pregnancy complication prevention.[28–32]

The local endometrium therapy is ongoing for several years.

Positive outcome was found after the intrauterine administration of autologous peripheral blood mononuclear cells (PBMCs)[33] especially when pretreated with corticotropin-releasing hormone (CRH) that acts by regulating apoptosis of activated T-lymphocytes at the implantation site.[34]

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.[35] Gleicher' papers on flushing uterus cavity with growth factors before the embryo transfer have proposed granulocyte colony-stimulating factor (G-CSF) as the treatment of implantation failure and repeated miscarriages.[36]

This type of therapy is recommended in order to increase the implantation rate and decrease the miscarriage rate. 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, miscarriage rate, by flushing uterus cavity with autologous platelet-rich plasma (PRP) in preparation for the implantation during IVF process.[37] Our group has, so far, treated 25 patients with that technology and has achieved a significant improvement of the implantation rates and in reducing the number of abortions. This therapy delivers biological growth factors, PDGF, TGF-β, VEGF, insulin-like growth factor I, epidermal growth factor (EGF) and epithelial cell growth factor to the endometrium. It is well known that autophagy is involved in the endometrial cell cycle affecting apoptosis and is most prominent during the late secretory phase. 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.[38]

Embryo

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.[39–41] 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 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.[42]

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.[43]

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.[44,45]

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.

Oogenesis

Evaluation of the oocytes 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.[46]

Better predictors, the birefringence properties of the meiotic spindle and the zona pellucida are indicative of good health of the oocyte.[47] 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.[48] Oocyte quality can be assessed by the measurement of oocyte oxygen consumption.[49]

Spermatogenesis

The quality spermatogenesis is the condition for formation of a good embryo. The development of the advanced diagnostic techniques and the semen choice are based not only on the morphological assessment [defragmentation, magnetic-activated cell sorting (MACS)] provide a choice of better quality sperm. The methods of improving conditions 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 oxydoreductive 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.[50] Similarly, centrosome integrity is critical for successful fertilization and embryo development. There are studies that have described an association between sperm with DNA damage and a history of recurrent miscarriage.[51]

Advanced Therapy

Magnetic-activated cell sorting 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.[52]

A number of conditions 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.[53]

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.[54]

Their dormant status is characterized by communication with surrounding granulosa cells and numerous mechanical and chemical factors controlling 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.[53] 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 existing ovarian primordial oogonia. The therapy with mesenchymal stem cells has led to the recovery features of oocytes after the chemotherapy-induced insufficiency.[55] The animal experiments by the *in vitro* therapy with developed stem cells have led to the birth of live offspring without abnormalities.[56] The other group 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 retransplantation of ovaries with support of PRP have been published.[57] 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, pronuclear or oocyte transfer.[58–60] The augmentation of autologous mitochondria carries a potential treatment. Our team has inaugurated the attempt of the energy mitochondrial boosting with ovarian hyperintensive 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.[61,62]

Instead of the 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 peri-implantation periods) the therapy based on the subcellular and genetic level by applying the latest biotechnological procedures.

Orcid

Aleksandar Ljubić https://orcid.org/0000-0003-0647-316X

References

1. Nicolaides K. A model for a new pyramid of prenatal care based on the 11 to 13 weeks' assessment. Prenat Diagn 2011;31(1):3–6. DOI: 10.1002/pd.2685.
2. Wright D, Syngelaki A, Bradbury I, et al. First-trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemicaltesting. Fetal Diagn Ther 2014;35(2):118–126. DOI: 10.1159/000357430.
3. Sonek JD, Cuckle HS. What will be the role of first-trimester ultrasound if cell-free DNA screening for aneuploidy becomes routine? Ultrasound Obstet Gynecol 2014;44(6):621–630. DOI: 10.1002/uog.14692.
4. Renna MD, Pisani P, Conversano F, et al. Sonographic markers for early diagnosis of fetal malformations. World J Radiol 2013;5(10):356–371. DOI: 10.4329/wjr.v5.i10.356.
5. Stoll C, Clementi M, Euroscan study group Prenatal diagnosis of dysmorphic syndromes by routine fetal ultrasound examination across Europe. Ultrasound Obstet Gynecol 2003;21(6):543–551. DOI: 10.1002/uog.125.
6. Rydberg C, Tunón K. Detection of fetal abnormalities by second-trimester ultrasound screening in a non-selected population. Acta Obstet Gynecol Scand 2017;96(2):176–182. DOI: 10.1111/aogs.13037.
7. Datta MR, Raut A. Efficacy of first-trimester ultrasound parameters for prediction of early spontaneous abortion. Int J Gynaecol Obstet 2017;138(3):325–330. DOI: 10.1002/ijgo.12231.
8. Wu Y, He J, Guo C, et al. Serum biomarker analysis in patients with recurrent spontaneous abortion. Mol Med Rep 2017;16(3):2367–2378. DOI: 10.3892/mmr.2017.6890.
9. Greco E, Lange A, Ushakov F, et al. Prediction of spontaneous preterm delivery from endocervical length at 11 to 13 weeks. Prenat Diagn 2011;31(1):84–89. DOI: 10.1002/pd.2640.
10. Conde-Agudelo A, Romero R. Predictive accuracy of changes in transvaginal sonographic cervical length over time for preterm birth: a systematic review and metaanalysis. Am J Obstet Gynecol 2015;213(6):789–801. DOI: 10.1016/j.ajog.2015.06.015.
11. Poon LC, Nicolaides KH. Early prediction of preeclampsia. Obstet Gynecol Int 2014;1:1–11. DOI: 10.1155/2014/297397.
12. Akolekar R, Syngelaki A, Sarquis R, et al. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11–13 weeks. Prenat Diagn 2011;31(1):66–74. DOI: 10.1002/pd.2660.
13. Mihailovič M, Cvetkovč M, Ljubič A, et al. Selenium and malondialdehyde content and glutathione peroxidase activity in maternal and umbilical cord blood and amniotic fluid. Biol Trace Elem Re 2000;73(1):47–54. DOI: 10.1385/BTER:73:1:47.
14. Ćetković A, Miljic D, Ljubić A, et al. Plasma kisspeptin levels in pregnancies with diabetes and hypertensive disease as a potential marker of placental dysfunction and adverse perinatal outcome. Endocr Res 2012;37(2):78–88. DOI: 10.3109/07435800.2011.639319.
15. Giguère Y, Charland M, Bujold E, et al. Combining biochemical and ultrasonographic markers in predicting preeclampsia: a systematic review. Clin Chem 2010;56(3):361–375. DOI: 10.1373/clinchem.2009.134080.

16. Duley L, Henderson-Smart DJ, Meher S, et al. Antiplatelet agents for preventing pre-eclampsia and its complications. Cohrane Database Syst Rev 2007;2:CD0004659. DOI: 10.1002/14651858.CD004659.pub2.
17. Karagiannis G, Akolekar R, Sarquis R, et al. Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11-13 weeks. Fetal Diagn Ther 2011;29(2):148–154. DOI: 10.1159/000321694.
18. Poon LCY, Karagiannis G, Stratieva V, et al. First-trimester prediction of macrosomia. Fetal Diagn Ther 2011;29(2):139–147. DOI: 10.1159/000318565.
19. Baltaci V, Baltaci E. Genetic aspects of recurrent miscarriages. JSM Invitro Fertil 2016;1:1002.
20. Chabra S. Estimates of perinatal death: a global initiative!. J Perinat 2017;37(11):1248. DOI: 10.1038/jp.2017.81.
21. Rolnik DL, O'Gorman N, Roberge S, et al. Early screening and prevention of preterm pre-eclampsia with aspirin: time for clinical implementation. Ultrasound Obstet Gynecol 2017;50(5):551–556. DOI: 10.1002/uog.18899.
22. Gregory EC, MacDorman MF, Martin JA. Trends in fetal and perinatal mortality in the United States, 2006-2012. NCHS Data Brief 2014;169:1–8.
23. Ananth CV, Keyes KM, Wapner RJ. Pre-eclampsia rates in the United States, 1980-2010: Age-period-cohort analysis. BMJ 2013;347(nov07 15):f6564. DOI: 10.1136/bmj.f6564.
24. Ljubić A. Inverted pyramid of prenatal care - is it enough? Should it be -extended inverted pyramid of prenatal care? J Perinat Med 2017;pii(7):/j/jpme.ahead-of-print/jpm-2016-0427/jpm-2016-0427.xml 10.1515/jpm-2016-0427.
25. Urato AC, Norwitz ER. A guide towards pre-pregnancy management of defective implantation and placentation. Best Pract Res Clin Obstet Gynaecol 2011;25(3):367–387. DOI: 10.1016/j.bpobgyn.2011.01.003.
26. Acharya S, Yasmin E, Balen AH. The use of a combination of pentoxifylline and tocopherol in women with a thin endometrium undergoing assisted conception therapies—a report of 20 cases. Hum Fertil (Camb) 2009;12(4):198–203. DOI: 10.3109/14647270903377178.
27. Shen MS, Wang CW, Chen CH, et al. New horizon on successful management for a woman with repeated implantation failure due to unresponsive thin endometrium: use of extended estrogen supplementation. J Obstet Gynaecol Res 2013;39(5):1092–1094. DOI: 10.1111/j.1447-0756.2012.02070.x.
28. Stephenson MD, Fluker MR. Treatment of repeated unexplained in vitro fertilization failure with intravenous immunoglobulin: a randomized, placebo-controlled Canadian trial. Fertil Steril 2000;74(6):1108–1113. DOI: 10.1016/S0015-0282(00)01622-8.
29. Stern C, Chamley L, Norris H, et al. A randomized, double-blind, placebo-controlled trial of heparin and aspirin for women with in vitro fertilization implantation failure and antiphospholipid or antinuclear antibodies. Fertil Steril 2003;80(2):376–383. DOI: 10.1016/S0015-0282(03)00610-1.
30. Boomsma CM, Keay SD, Macklon NS. Peri-implantation glucocorticoid administration for assisted reproductive technology cycles. Cochrane Data Base Syst Rev 2007. CD005996.
31. Gelbaya TA, Kyrgiou M, Li TC, et al. Low-dose aspirin for in vitro fertilization: a systematic review and meta-analysis. Hum Reprod Update 2007;13(4):357–364. DOI: 10.1093/humupd/dmm005.
32. Mekinian A, Cohen J, Alijotas-Reig J, et al. Unexplained recurrent miscarriage and recurrent implantation failure: Is there a place for immunomodulation? Am J Reprod Immunol 2016;76(1):8–28. DOI: 10.1111/aji.12493.
33. Yu N, Zhang B, Xu M, et al. Intrauterine administration of autologous peripheral blood mononuclear cells (PBMCs) activated by hCG improves the implantation and pregnancy rates in patients with repeated implantation failure: a prospective randomized study. Am J Reprod Immunol 2016;76(3):212–216. DOI: 10.1111/aji.12542.
34. Makrigiannakis A, BenKhalifa M, Vrekoussis T, et al. Repeated implantation failure: a new potential treatment option. Eur J Clin Invest 2015;45(4):380–384. DOI: 10.1111/eci.12417.
35. Wurfel W. Treatment with granulocyte colony-stimulating factor in patients with repetitive implantation failures and/or recurrent spontaneous abortions. J Reprod Immunol 2015;108:123–135. DOI: 10.1016/j.jri.2015.01.010.
36. Gleicher N, Vidali A, Barad DH. Successful treatment of unresponsive thin endometrium. Fertil Steril 2011;95(6):2123. DOI: 10.1016/j.fertnstert.2011.01.143.
37. Chang Y, Li J, Chen Y, et al. Autologous platelet-rich plasma promotes endometrial growth and improves pregnancy outcome during in vitro fertilization. Int J Clin Exp Med 2015;8:1286–1290.
38. Sumarac-Dumanovic M, Apostolovic M, Janjetovic K, et al. Downregulation of autophagy gene expression in endometria from women with polycystic ovary syndrome. Mol Cell Endocrinol 2016;440:116–124. DOI: 10.1016/j.mce.2016.11.009.
39. Schoolcraft WB, Fragouli E, Stevens J, et al. Clinical application of comprehensive chromosomal screening at the blastocyst stage. Fertil Steril 2010;94(5):1700–1706. DOI: 10.1016/j.fertnstert.2009.10.015.
40. Schoolcraft WB, Treff NR, Stevens JM, et al. Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism microarray-based comprehensive chromosome screening in infertile patients. Fertil Steril 2011;96(3):638–640. DOI: 10.1016/j.fertnstert.2011.06.049.
41. Bisignano A, Wells D, Harton G, et al. PGD and aneuploidy screening for 24 chromosomes: advantages and disadvantages of competing platforms. Reprod Biomed Online 2011;23(6):677–685. DOI: 10.1016/j.rbmo.2011.05.017.
42. Wong CC, Loewke KE, Bossert NL, et al. Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. Nat Biotechnol 2010;28(10):1115–1121. DOI: 10.1038/nbt.1686.
43. Kirkegaard K, Ahlström A, Ingerslev HJ, et al. Choosing the best embryo by time lapse versus standard morphology. Fertil Steril 2015;103(2):323–332. DOI: 10.1016/j.fertnstert.2014.11.003.
44. Yang Z, Zhang J, Salem SA, et al. Selection of competent blastocysts for transfer by combining time-lapse monitoring and array CGH testing for patients undergoing preimplan- tation genetic screening: a prospective study with sibling oocytes. BMC Med Genomics 2014;7(1):38. DOI: 10.1186/1755-8794-7-38.
45. Armstrong S, Arroll N, Cree LM, et al. Time-lapse systems for embryo incubation and assessment in assisted reproduction. Cochrane Database Syst Rev 2015;2:CD011320. DOI: 10.1002/14651858.CD011320.pub2.
46. Seli E, Robert C, Sirard MA. OMICS in assisted reproduction: possibilities and pitfalls. Mol Hum Reprod 2010;16(8):513–530. DOI: 10.1093/molehr/gaq041.
47. Menezo Y, Elder K, Benkhalifa M, et al. DNA methylation and gene expression in IVF. Reprod Biomed Online 2010;20(6):709–710. DOI: 10.1016/j.rbmo.2010.02.016.
48. Nagy ZP, Jones-Colon S, Roos P, et al. Metabolomic assessment of oocyte viability. Reprod Biomed Online 2009;18(2):219–225. DOI: 10.1016/S1472-6483(10)60259-3.
49. Tejera A, Herrero J, de Los Santos MJ, et al. Oxygen consumption is a quality marker for human oocyte competence conditioned by ovarian stimulation regimens. Fertil Steril 2011;96(3):618–623. DOI: 10.1016/j.fertnstert.2011.06.059.
50. Evgeni E, Byron A. Human sperm DNA fragmentation and its correlation with conventional semen parameters. J Reprod Infertil 2014;15(1):2–14.
51. Leach M, Aitken R, Sacks G, et al. Fragmentation abnormalities in men from couples with a history of recurrent miscarriage. Aust N Z J Obstet Gynaecol 2015;55(4):379–383. DOI: 10.1111/ajo.12373.
52. Gil M, Shalom V, Carreras S. Sperm selection using magnetic activated cell sorting (MACS) in assisted reproduction: a systematic review and meta-analysis. J Assist Reprod Genet 2013;30(4):479–485. DOI: 10.1007/s10815-013-9962-8.
53. Cheng Y, Feng Y, Jansson L, et al. Actin polymerization-enhancing drugs promote ovarian follicle growth mediated by the Hippo

signaling effector YAP. FASEB J 2015;29(6):2423–2430. DOI: 10.1096/fj.14-267856.
54. Johnson J, Canning J, Kaneko T, et al. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004;428(6979):145–150. DOI: 10.1038/nature02316.
55. Afifi N, Reyad O. Role of mesenchymal stem cell therapy in restoring ovarian function in a rat model of chemotherapy-induced ovarian failure: a histological and immunohistochemical study. Egypt J Histol 2013;36(1):114–126. DOI: 10.1097/01.EHX.0000423979.18253.10.
56. Hayashi K, Ogushi S, Kurimoto K, et al. Offspring from oocytes derived from in vitro primordial germ cell–like cells in mice. Science 2012;338(6109):971–975. DOI: 10.1126/science.1226889.
57. Callejo J, Salvador C, González-Nuñez S, et al. Live birth in a woman without ovaries after autograft of frozen-thawed ovarian tissue combined with growth factors. J Ovarian Res 2013;6(1):33. DOI: 10.1186/1757-2215-6-33.
58. Smeets HJ. Preventing the transmission of mitochondrial DNA disorders: selecting the good guys or kicking out the bad guys. Reprod Biomed Online 2013;27(6):599–610. DOI: 10.1016/j.rbmo.2013.08.007.
59. Amato P, Tachibana M, Sparman M, et al. Three-parent in vitro fertilization: gene replacement for the prevention of inherited mitochondrial diseases. Fertil Steril 2014;101(1):31–35. DOI: 10.1016/j.fertnstert.2013.11.030.
60. Mitalipov S, Wolf DP. Clinical and ethical implications of mitochondrial gene transfer. Trends Endocrinol Metab 2014;25(1):5–7. DOI: 10.1016/j.tem.2013.09.001.
61. Kawamura K, Cheng Y, Suzuki N, et al. Hippo signaling disruption and AKT stimulation of ovarian follicles for infertility treatment. PNAS 2013;110(43):17474–17479. DOI: 10.1073/pnas.1312830110.
62. Ljubić A, Abazović D, Vučetić D, et al. Autologous ovarian in vitro activation with ultrasound-guided orthotopic re-transplantation. Am J Clin Exp Obstet Gynecol 2017;4:51–57. (In press).