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FREEZE-ALL STRATEGY: PREGNANCY RATE, OBSTETRICAL PROGNOSIS AND ETHICAL CONSIDERATIONS – REVIEW EVALUATING 278.000 NEWBORNS AFTER ART

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ABSTRACT:
INTRODUCTION: CONTROLLED OVARIAN HYPERSTIMULATION (COH) MAY HAVE A NEGATIVE IMPACT UPON THE ENDOMETRIAL ENVIRONMENT, THUS, A "FREEZE-ALL" (FET) STRATEGY WAS CONSIDERED.
METHODS: REVIEW INCLUDING 44 STUDIES EVALUATING 278.000 NEWBORNS AFTER ART, COMPARING THE RESULTS OF THE CLASSIC VERSUS FREEZE-ALL STRATEGY.
RESULTS: THE CUMULATIVE LIVE BIRTH RATE (LBR) AND PREGNANCY RATE (PR) WERE SIGNIFICANTLY HIGHER IN THE "FREEZE-ALL" (FET) VERSUS ET (LBR: 60.55% VS. 45%, PR: RR 1.30 [CI 95%]). FET IS ASSOCIATED WITH A LOWER OBSTETRIC RISK [CI 95%]: THE FETUS HAS A LOWER RISK OF BEING SMALL FOR GESTATIONAL AGE RR: 0.59, LOW GESTATIONAL WEIGHT RR: 0.74, PREMATURE BIRTH RR: 0.74 BUT MORE INCREASED RISK OF CESAREAN RR: 1.10 AND LARGE FOR GESTATIONAL AGE RR: 1.49. WITH REGARD TO THE RISKS OF ANTEPARTUM HAEMORRHAGE, PLACENTA PRAEVIA, PERINATAL MORTALITY, CONGENITAL ANOMALIES AND SPONTANEOUS ABORTION RATE, THERE ARE NO CONSISTENT FINDINGS: SOME STUDIES CONCLUDED THAT THE RISK IS LOWER IN FET (0.67, 0.68, 0.8 AND, RESPECTIVELY 0,83), BUT MOST CONSIDER THAT THERE IS NO SIGNIFICANT STATISTICAL DIFFERENCE. GESTATIONAL HYPERTENSION IS CONTROVERSIAL, SOME STUDIES HAVE FOUND THAT IT IS MORE COMMONLY LINKED TO FET (RR: 1.29), BUT THE RESULTS ARE INSIGNIFICANTLY STATISTICALLY DIFFERENT. HOWEVER, FET IS AN INDEPENDENT RISK FACTOR FOR PLACENTA ACCRETA, 3 TIMES HIGHER THAN ET. MONOZYGOTIC MONOCHORIONIC PREGNANCY AFTER SINGLE-EMBRYO IS LOWER IN FET IN GENERAL (0.8%), BUT MATERNAL AGE BELOW 35 YEARS IS A RISK FACTOR IN FET CYCLES.
CONCLUSIONS: "FREEZE-ALL" IS AN ELIGIBLE PROTOCOL.

KEY WORDS: CRYOPRESERVATION, EMBRYO TRANSFER, FREEZE-ALL, IN VITRO FERTILIZATION, OBSTETRIC COMPLICATIONS

INTRODUCTION

Assisted Reproductive Techniques (ART) generally involve the transfer of a fresh embryo (ET) during the controlled ovarian hyperstimulation cycle (COH) and the subsequent transfer of one or more cryopreserved (frozen and then thawed) embryos (FET) into the following cycles. The basic principle of infertility treatment, in short, is the use of products that modify hormonal levels in the body and determine and control the process of ovarian follicular growth and maturation of the oocytes resulting from these follicles. These oocytes are than extracted by transvaginal puncture of the ovaries under anesthesia, placed in the embryology lab in contact with the sperm[11] and so there will result a number of embryos (3 days old) or blastocysts (5 days old). Fertlization can be made classically, called in vitro fertilization (IVF) or by intracytoplasmic sperm injection,

[11] Marcu, D; Brat, O; Spinu, D, Radulescu, A; Farca, C; Mischian, D. Penile prothesis-a viable solution for erectile dysfunction refractary to conservatory therapy. Romanian Journal of Military Medicine 2015;118(3): 33-39.

especially when there are male pathology involved [12] [13] [14] [15] [16] (ICSI). At this point, the patient, under the guidance of the attending physician and the embryologist, has the following options: the transfer of a fresh embryo/ blastocyst or the cryopreservation of all of them and transfer them in subsequent cycles.

Ovarian hyperstimulation treatment has positive effects on the ovaries but has been shown to also have a negative impact on the endometrial uterine environment, which may affect the implantation or the development of pregnancy[17]. Thus, a "freeze-all" strategy has been considered by many clinics around the world[18]. What is this strategy basically? Freezing all the resulting embryos and transferring them sequentially, in the next cycles, leaving the body to "recover" after ovarian hyperstimulation. So the "freeze-all" approach consists of two steps and can be schematized as follows: in the first menstrual cycle the focus is on the ovaries: ovarian stimulation, follicle and oocyte development, puncture, oocyte harvesting. In step 2, in the next cycle, emphasis is on the uterus: developing and analyzing the endometrium, improving and monitoring the place where the pregnancy will be implanted[19].

OBJECTIVE

In infertility, an event of maximum emotional intensity is represented by the moment of embryo transfer, the transfer of the resulting embryo to the mother's uterus. Most couples hope that this is the moment when their struggle has come to an end and they will find out that they are parents. Unfortunately, on average, only 1 out of 3 embryo transfers will result in the birth of a living fetus (36%)[20]. It is an interesting subject, because both couples and us as physicians, feel involuntary disappointment at the moment when a pregnancy is not obtained after the transfer of the embryo.

[12] Bratu, O; Spinu, D; Oprea, I; Popescu, R; Marcu, D; Farcas, C; Dinu, M; Mischianu, D. Complications of radical retropubic prostatectomy-our experience. Romanian Journal of Military Medicine 2015;118(3):23-25.
[13] Bratu, O; Oprea, I; Marcu, D; Spinu, D; Niculae, A; Geavlete, B; Mischianu, D. Erectile dysfunction post-radical prostatectomy – a challenge for both patient and physician. Journal of Medicine and Life 2017;10(1):13-18.
[14] Marcu, D; Bratu, O; Spinu, D; Oprea, I; Niculae, A; Mischianu, D. Therapeutic approaches in premature ejaculation. Modern Medicine 2016;23(4): 270-278.
[15] Minciună, V; Crețu, O; Spînu, D; Marcu, D; Bratu, O. The impact of testicular cancer on fertility - literature review. Modern Medicine 2017;24(4):195-198.
[16] Marcu, D; Bratu, O; Spinu, D; Oprea, I; Niculae, A; Mischianu, D. Therapeutic approaches in premature ejaculation. Modern Medicine 2016;23(4): 270-278.
[17] Mehedintu, C; Bratila, E; Brinduse, LA; Cirstoiu, MM; Berceanu, C; Bordea, A; Comandasu, DE; Carp Veliscu, A; Bratu, O; Sava, C; Bumbu, AG. Controlled ovarian stimulation with urinary gonadotrophins and recombinant gonadotrophins in current practice. Rev Chim (Bucharest) 2018;69(12): 3611-3615.
[18] Chang, JC; Chen, MJ; Guu, HF; Chen, YF; Yi, YC; Kung, HF; Chen, LY; Chou, MM. Does the "freeze-all" policy allow for a better outcome in assisted reproductive techniques than the use of fresh embryo transfers? - A retrospective study on cumulative live birth rates. Taiwan J Obstet Gynecol. 2017;56(6):775-780. doi:10.1016/j.tjog.2017.10.013
[19] Shapiro, BS; Daneshmand, ST; Garner, FC; Aguirre, M; Hudson, C and Thomas, S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen thawed embryo transfer in normal responders. Fertil Steril 2011;96:344–348.
[20] Silea, C; Cucu, IA; Zarnescu, O; Stoian, AP; Motofei, IG; Bratu, OG; Pircalabioru, GG; Chifiriuc, MC. Influence of age on sperm parameters in men with suspected infertility. Rom Biotechnol Lett 2019;24(1):82-90.

The main purpose of the study is to improve the chances of achieving a pregnancy in in vitro fertilization programs by improving the embryo transfer method[21]. At the same time, the study aims to reduce maternal and fetal risks associated with pregnancy obtained from assisted human reproduction (ART) programs by analyzing comparatively the pregnancies obtained after the transfer of fresh embryos versus cryopreserved embryos[22].

MATERIALS AND METHODS

We performed a review including 44 studies evaluating 278.000 newborns after ART, comparing the results of the classic versus freeze-all strategy. In this paper, we analyze and compare, based on international studies, the live birth rates and pregnancy rates of fresh embryo transfer and defrosted embryo transfer, the obstetric complication rates in both categories, the rate of abortion, small for gestational age or macrosomia, hypertension, chromosomal anomalies, placenta accreta, monochorionic twin pregnancy after single embryotransfer, in order to quantify the advantages and disadvantages of the pregnancies obtained after fresh and cryopreserved embryotransfer. In addition to this, special infertile groups are taken into account, as low ovarian reserve patients and endometriosis patients, where the issue of fertility preservation and social freezing is raised as well, alongside with the ethical implications of cryopreservation that one must always have in mind while performing freezing of the embryos.

RESULTS

1. Pregnancy rates

There are currently studies that concluded that the life birth rate is higher if all embryos are frozen after fertilization, and they will be transferred to subsequent cycles (LBR: 60.55% vs. 45%)[23]. The cumulative pregnancy rate was also significantly higher in the “freeze-all” group (FET) versus fresh embryo transfer group (ET)[24] (PR: RR 1.30 [95% CI]).

This is because during the ovarian hyperstimulation cycle that results in oocyte production, the medication has a negative influence on the quality of the endometrium[25], thus affecting the implantation, and so influencing the number of pregnancies[26]. Uterine contractility can also be

[21] Weinerman, R and Mainigi, M. Why we should transfer frozen instead of fresh embryos: the translational rationale. Fertil Steril 2014;102:10–18.

[22] Spijkers, S; Lens, JW; Schats, R; Lambalk, CB. Fresh and Frozen-Thawed Embryo Transfer Compared to Natural Conception: Differences in Perinatal Outcome. Gynecol Obstet Invest 2017;82:538–546.

[23] Roque, M, Valle, M, Kostolias, A, Sampaio, M, Geber, S. Freeze-all cycle in reproductive medicine: current perspectives. JBRA Assist Reprod 2017;21(1):49-53. doi: 10.5935/1518-0557.20170012. PMID: 28333033.

[24] Roque, M, Valle, M, Guimarães, F, Sampaio, M, and Geber, S. Freeze-all policy: fresh vs. frozen-thawed embryo transfer. Fertil Steril 2015; 103: 1190–1193.

[25] Wong, K, van Wely, M, Mol, F, Repping, S, Mastenbroek, S. Fresh versus frozen embryo transfers in assisted reproduction. Cochrane Database of Systematic Reviews 2017, Issue 3. Art. No.: CD011184. DOI: 10.1002/14651858.CD011184.pub2

[26] Roy, TK; Bradley, CK; Bowman, MC; and McArthur, SJ. Single-embryo transfer of vitrified-warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes compared with fresh transfers. Fertil Steril 2014; 101: 1294–1301.

increased in the same cycle, and the implantation window is sometimes missed at the time of transfer[27].

2. Placentation and associated obstetric complications

In addition to the number of pregnancies that are lower in the fresh embryo group, it appears that ovarian hyperstimulation drugs also affect the development of pregnancy, placenta is altered in the case of ET in the same stimulation cycle[28] and so many obstetric complications associated with this process occur[29]. Thus, in the case of frozen embryos, a better pregnancy prognosis [95% CI] was observed: the fetus had a lower risk of being small for gestational age RR: 0.59, reduced gestational weight RR: 0.74, premature delivery RR: 0.74, but higher risk of RR: 1.10 cesarean and larger for gestational age RR: 1.49[30].

Gestational hypertension is controversial, some studies have found that it is more commonly linked to FET (RR: 1.29), but the results are insignificantly statistically different[31].

With regard to the risks of antepartum haemorrhage[32], placenta praevia[33], perinatal mortality[34], congenital anomalies[35] and spontaneous abortion rate[36], there are no consistent findings: some studies concluded that the risk is lower in FET (0.67, 0.68, 0.8 and, respectively 0.83), but most of the studies consider that there is no significant statistical difference[37] .

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[27] Maheshwari, A; Pandey, S; Shetty, A; Hamilton, M; and Bhattacharya, S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: A systematic review and meta-analysis. Fertil Steril. 2012; 98: 368–377.

[28] Aflatoonian, A; Karimzadeh Maybodi, MA; Aflatoonian, N; et al. Perinatal outcome in fresh versus frozen embryo transfer in ART cycles. International Journal of Reproductive Biomedicine. 2016;14(3):167-172.

[29] Imudia, AN; Awonuga, AO; Kaimal, AJ; Wright, DL; Styer, AK; and Toth, TL. Elective cryopreservation of all embryos with subsequent cryothaw embryo transfer in patients at risk for ovarian hyperstimulation syndrome reduces the risk of adverse obstetric outcomes: A preliminary study. Fertil Steril. 2013; 99: 168–173.

[30] Roque, M; Lattes, K; Serra, S; Sola, I; Geber, S; Carreras, R; et al. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. Fertil Steril. 2013;99:156–162.

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[33] Ozgur, K; Berkkanoglu, M; Bulut, H; Humaidan, P; Coetzee, K. Perinatal outcomes after fresh versus vitrified - warmed blastocyst transfer: retrospective analysis. DOI: http://dx.doi.org/10.1016/j.fertnstert.2015.06.031

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[35] Ginström Ernstad, E; Wennerholm, UB; Khatibi, A; Petzold, M; Bergh, C. Neonatal and maternal outcome after frozen embryo transfer: increased risks in programmed cycles. Am J Obstet Gynecol. 2019 Mar 22. pii: S0002-9378(19)30487-9. doi: 10.1016/j.ajog.2019.03.010. [Epub ahead of print].
https://www.ncbi.nlm.nih.gov/pubmed/30910545

[36] Maheshwari, A; Pandey, S; Amalraj, Raja, E; Shetty, A; Hamilton, M; Bhattacharya, S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? Hum Reprod Update. 2018 Jan 1;24(1):35-58. doi: 10.1093/humupd/dmx031. https://www.ncbi.nlm.nih.gov/pubmed/29155965

[37] Roque, M; Valle, M; Kostolias, A; Sampaio, M; Geber, S. Freeze-all cycle in reproductive medicine: current perspectives. JBRA Assist Reprod. 2017 Feb 1;21(1):49-53. doi: 10.5935/1518-0557.20170012. PMID: 28333033

3. The risk of fetal macrosomia and the rate of cesarean operations

Fetus resulting from cryopreserved embryo transfer are at risk of being macrosomes (large for gestational age) RR: 1.49 and at higher risk of posterm delivery, and mothers have an increased incidence of birth by cesarean section RR: 1.10[38].

4. Embryo transfer or blastocyst?

Generally, studies suggest that blastocyst transfer has a higher rate of achieving a pregnancy[39]. However, there are many contradictory but we should mention low quality studies due to the lack of a clear design. Some studies suggest that the transfer of cryopreserved blastocysts would be the optimal option to reach the largest number of live newborns, but other studies point out that these findings would not be statistically significant[40]. To assess whether the cumulative pregnancy rate and live birth rate is influenced by intrauterine embryo transfer at the cleavage stage (day 2, 3) or blastocyst (day 5, 6), we used a Cochrane meta-analysis that included 27 randomized clinical trials involving 4031 women[41]. Comparing the fresh group, the live newborn rate after fresh blastocyst was higher than after fresh embryo transfer: (OR) 1.48, 95% confidence interval (CI) 1.20 to 1.82, 13 RCTs, 1630 women, I2 = 45%, low quality records)[42]. There were no differences in the cumulative pregnancy rate after a single ovarian puncture between the fresh and the cryopreserved group (OR 0.89, 95% CI 0.64 to 1.22, 5 RCTs, 632 women, I2 = 71%, very low quality evidence). Comparing the blastocyst group, the clinical pregnancy rate was higher for fresh blastocysts (OR 1.30, 95% CI 1.14 to 1.47, 27 RCTs, 4031 women, I2 = 56%, moderate quality evidence). There were no significant differences in abortion rate or multiple group loads (low quality evidence). The main problems encountered were the lack of standard randomization and analysis methods and the high risk of bias. Studies are needed to analyze the same results first (eg cumulative pregnancy rate or live birth rate or pregnancy rate per transfer) and including and comparing patients with the same characteristics, knowing that ovarian reserve and age of the patient are essential features that can dramatically alter the results of assisted human reproductive techniques, independent of the transfer method.

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[41] Alviggi, C; Conforti, A; Carbone, IF; Borrelli, R; de Placido, G; Guerriero, S. Influence of cryopreservation on perinatal outcome after blastocyst - vs cleavage-stage embryo transfer: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2018 Jan; 51(1):54-63. doi: 10.1002/uog.18942.
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[42] Wang, X; Du, M; Guan, Y; Wang, B; Zhang, J; Liu, Z. Comparative neonatal outcomes in singleton births from blastocyst transfers or cleavage-stage embryo transfers: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2017 May 4;15(1):36. doi: 10.1186/s12958-017-0255-4.
https://www.ncbi.nlm.nih.gov/pubmed/28472983

5. Congenital fetal abnormalities

Regarding the risk of fetal abnormalities[43], it is known that fetuses obtained by assisted human reproductive techniques may statistically have a higher risk of congenital malformation than those in the population of spontaneously obtained pregnancies[44]. This is mainly due to the fact that, in the general population, pregnancies with various malformations often stop spontaneously in their incipient stages, while IVF / ICSI pregnancies are medically supported and closely monitored. However, the risk of pregnancy with fetal abnormalities is no longer increased but is similar to that of the general population[45] , in the group of patients with cryopreserved embryo transfer[46]. We can attribute this to the fact that not all embryos survive to the freezing / thawing process, so that a "natural selection" of the highest quality embryos will be achieved, which will then be implanted and thus will result in pregnancies with healthy fetuses. Several studies have compared the risk of birth defects associated with defective blastogenesis in IVF pregnancies with fresh ET and frozen versus spontaneous pregnancies. It has been shown that the risk may be 3 times higher in the case of the transfer of fresh embryos to spontaneous pregnancies, but in the case of cryopreserved and defrosted embryo transfer this risk is not increased, but it is the same as in the spontaneously obtained pregnancies[47]. In addition to this, cryopreservation provides the possibility of preimplantation genetic screening. It has been observed that the biopsy should be performed in the blastocyst stage, not to interfere with the normal development of the embryo. So, in the case of couples at risk of genetic syndromes, conduct should be genetic testing on day 5 to 6 followed by cryopreservation of the blastocyst and then making a decision on implantation in the next cycle.

6. Monochorionic monozygotic twin pregnancy risk after single fresh or cryopreserved embryo transfer

Even after single embryo transfer, assisted human reproduction presents a 2-fold higher risk of monochorionic twin pregnancy compared to spontaneous pregnancies. To evaluate this, we made a review of retrospective cohort studies[48]. Although it was postulated that the culture medium

[43] Halliday, J.L., Ukoumunne, O.C., Baker, H.W., Breheny, S., Jaques, A.M., Garrett, C. et al. Increased risk of blastogenesis birth defects, arising in the first 4 weeks of pregnancy, after assisted reproductive technologies. Hum Reprod. 2010; 25: 59–65.

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[46] Maheshwari, A., Pandey, S., Shetty, A., Hamilton, M., and Bhattacharya, S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: A systematic review and meta-analysis. Fertil Steril. 2012; 98: 368–377.

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[48] Knopman, J; Krey, LC; Lee, J; Fino, ME; Novetsky, AP; Noyes, N. Monozygotic twinning: an eight-year experience at a large IVF center. Fertil Steril. 2010 Jul;94(2):502-10. doi: 10.1016/j.fertnstert.2009.03.064. Epub 2009 May 5.

would influence the monozygotic twin pregnancy rate after single embryo transfer and that implicitly the blastocysts, having 5 days in the culture medium, are at increased risk because they have been exposed for a longer time, the latest studies show that the culture medium do not influence the rate of monozygotic pregnancy, but instead, the quality of the embryo plays an important role[49]. Thus, the interesting discovery is that intrinsic factors rather than extrinsic factors influence the embryo's division process and the occurrence of the twin pregnancy after single transfer[50]. The highest rates of monochorionic monozygotic pregnancies were reported in single embryo transfer obtained with donated oocytes, so the superior quality oocytes (3.3%). Logistic regression analysis showed that patients under 35 years old with high quality recovered oocytes and increased number of day 3 or 5 embryos of superior quality had the highest monozygotic pregnancy rates (3.1%), regardless of use ICSI or assisted hatching [p <0.01].

Thus, the hypothesis regarding the damage to the pellucid area and hernia of the blastomere with the subsequent embryo division and the monozygotic pregnancy outcome is negated. Other studies have concluded that blastocyst transfer is a risk factor in fresh cycles (2.6% versus 1.2% fresh 3-day embryos), while maternal age under 35 is a risk factor in cryopreserved cycles. Generally, however, cycles using cryopreserved and thawed FET embryos had a lower rate of monozygotic pregnancies (0.8%). As a conclusion, monozygotic pregnancy rates are higher in ART, even after single transfer, especially in women under 35 years old. The risk is directly proportional to the number of available quality embryos, unaffected by the pellucid penetration procedures, and cryopreservation is a protective factor[51].

7. The risk of placenta accreta after cryopreserved embryo transfer

Assisted human reproduction poses a higher risk of placental pathology than spontaneous pregnancies, but an additional increased risk has been reported recently after the transfer of cryopreserved embryos (FET)[52]. For this reason, we performed a retrospective review to evaluate the correlation between cryopreserved and thawed embryo transfer and increased placenta accreta risk. We noticed that the prevalence of placenta accreta is 3 times higher after the transfer of cryopreserved embryos than after the transfer of fresh embryos (aOR, 3.2, P = 0.03). The physiopathological mechanisms are still in the debate[53]. The freezing method (slow freezing or

[49] Knopman, JM; Krey, LC; Oh, C; Lee, J; McCaffrey, C; Noyes, N. What makes them split? Identifying risk factors that lead to monozygotic twins after in vitro fertilization. Fertil Steril. 2014 Jul;102(1):82-9. doi: 10.1016/j.fertnstert.2014.03.039. Epub 2014 Apr 29.

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vitrification) seems to have no different effect, neither does the cleavage stage (embryo or blastocyst) or the fertilization technique used (FIV or ICSI). The risk appears to be related to the intrauterine environment, which is not hyperstimulation in cryopreserved transfer cycles. In women with placenta accreta, a lower level of estradiol and a 2 mm thinner endometrium have been reported. Another hypothesis is the implication of microRNA expression in the placenta, which may increase epigenome exposure to external influences. There have been 39 miRNAs that may be involved in this placental pathology. In conclusion, cryopreserved embryo transfer (FET) is an independent risk factor for placenta accreta, 3 times higher than for pregnancies using fresh embryos (ET), although the exact mechanism is not yet known[54]. The estradiol level, endometrial thickness and microARN appear to play an important role.

8. Ethical implications of human embryo cryopreservation

The development of assisted reproduction technologies (ART) has provided millions of infertile couples around the world with the hope of realizing their dream: having children. However, in most cases, more than one embryo result. Through cryopreservation techniques, the excessive number of embryos is kept until the family has achieved the number of children they want. Deciding what will happen with surplus embryos that have remained cryopreserved is of major ethical importance. The couple can either choose to dispose the embryos by defrosting them, or engage in compassionate transfer, donate them to other couples or give them for "adoption", or donate them for research[55]. Some couples simply "abandon" the embryos.

This subject is very sensitive, as embryos are not only simple transplanted tissues but have the potential to generate the life of a new human being[56]. In all the above-mentioned decisions the principles of bioethics must be taken into account. The principles of bioethics are: respect for autonomy (respect for the ability of the person to choose), non-maleficence (avoidance of harm), beneficence (the well-being of a person) and justice (equitable distribution of benefits and costs)[57]. In the decision-making process, there are specific laws that must be respected. We should mention: Human Reproduction and Embryology Societies laws[58], the Human Rights Declaration[59],[60] and the various available Civil and Penal codes that differ from country and state worldwide[61]. Christian religion does not support assisted reproductive techniques and especially not

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cryopreservation. Medical researchers by the other hand emphasize that embryonic stem cells have great potential to treat diseases such as cancer, genetic disorders and infertility.

One important aspect is concerning the fundamental right of people – the right to life – but from what point do we consider the product of conception as a human being with consciousness[62], sensitivity[63], the ability to feel the pain[64]? Neurologic, embryologic and biologic studies show that at the cell level the embryo has not the characteristics in order to consider it as a full righted human being. Is it ethical or not in this case to harvest stem cells, study embryos donated for research or destruction?[65] European legislation in this respect is not homogeneous[66], and Romanian legislation[67] is even more restrictive as the field of assisted human reproduction is under the legislation on organ transplantation[68].

An important point to remember is to strictly decide before starting any ART procedure what will happen with the surplus embryos and the "parents" to sign an informed consent and a contract concerning this issue, to avoid legal problems afterwards. A second point that I would like to highlight is that as doctors, we should explain the importance of the wider picture to the couple, taken into account donating the embryos for adoption or for research development[69]. It is not a simple decision to make but by emphasizing the concept of "beneficence," we could help improve treatments, relieve the suffering caused by different illness, or help other infertile couples to realize their dream family[70].

9. Pregnancy rate after fresh and cryopreserved ET in patients with low ovarian reserve

A particular category of patients with infertility problems is women with low ovarian reserve. The reason we assign a separate chapter is that their response to ovarian stimulation is poor, with few oocyte retrieved and of low quality.[71]. In this regard, we conducted a research with

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the objective of assessing whether the treatment protocol or the embryo transfer method influences IVF prognosis in patients with low ovarian reserve. We performed a review of retrospective cohort studies with a total of 8,556 stimulation cycles for approximately 3,000 patients with AMH <1.1 ng / mL[72],[73],[74],[75],[76].

Stimulating protocols were long or short, using GnRH antagonists, FSH, hMG, GnRH agonists, and included fresh (ET) or thaw (FET) embryotransfer. The implantation rates and cumulative pregnancy rates were similar (approximately 17%). Depending on the age of the patient, it was noted that under 35 years old, even after one transferred embryo the pregnancy rate was 33%, regardless of AMH. This rate decreased with age and required an increasing number of embryo transfers. According to this, it was observed that after 3 embryo transfer cycles, a cumulative pregnancy rate of 25.7% was observed in patients <40 years, including those with very low AMH, while in patients ≥40 years the cumulative pregnancy rate was 17.6% and in patients over 42 years old 7.4%. By dividing the patients into 2 similar mean age groups, only by AMH level (AMH 0.2-1ng / mL and AMH <0.2 ng / mL) the cumulative pregnancy rate was 20% after 5 stimulation cycles in both groups. Regarding the number of live newborns, it was noted that up to 35 years, there was no statistical difference between AMH levels between the group with and without live births. Over 35 years the AMH was significantly higher in the group with pregnancies with live newborns.

As a conclusion, AMH can not be used as an independent marker. In patients <35 years AMH is not a predictor, even with AMH <0.2ng / mL they have a good cumulative pregnancy rate. Age of the patient significantly influences the pregnancy rate, in patients ≥40 years, a higher AMH value shows a better prognosis. The number of cycles increases the chances of pregnancy in these patients, recommended to be at least 3 embryo transfers. Regarding the use of a particular treatment protocol, IVF or ICSI technique, and the pathology causing decreased ovarian reserve, there were no differences. Also, the use of fresh or cryopreserved embryos or blastocysts did not show statistically significant differences.

10. Patients with endometriosis – the need for social freezing

Endometriosis patients are at high risk of infertility[77]. Since endometriosis usually occurs at young ages, when the patient does not desire yet a pregnancy, the subject of fertility preservation

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is essential[78]. Progesterone treatment[79] proved its benefits on improvement of the symptoms, dimensions of the cysts and intraoperative conditions[80] [81] [82]. Endometriosis patients have an even higher risk of infertility and premature ovarian failure when the pathology begins at earlier ages[83], due to the reduction in ovarian reserve, both by the direct effect of ovarian endometriomas or pelvic[84] and follicular high inflammation factors but also following repeated surgery, especially if the lesions are bilateral[85]. To this it is added the peritoneal inflammatory effect of endometriosis, resulting in pelvic adherence syndrome, with tubal obstruction and ovulation impairment[86] [87].

The rate of pregnancy decreases directly proportional with the duration and severity of endometriosis, the time until the pregnancy is desired to happen and the need for repeated surgery[88]. In case of deep endometriosis, the symptomatology was correlated with the disease stage[89]. Early consultation with ART specialists to determine the need to use fertility preservation will increase the chances of this patients to eventually conceive at the wished moment. They must take into consideration age, ovarian reserve, the stage of endometriosis, IHC implants profile, the number of previous operations, the desire of procreation and the symptomatology[90]. In order to

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establish a correct diagnosis, a sonovaginography with ultrasound gel is essential [91]. Depending on age, ovarian reserve and the severity of endometriosis, one can opt for cryopreservation of ovarian tissue, oocytes or embryos[92]. The rate of pregnancy is highest after embryo preservation, but cryopreservation of oocytes gives the patient independence, as maybe at the moment they do not have a stable partner. The cryopreservation of ovarian tissue can be done during the laparoscopic procedure of diagnosis or treatment of endometriosis and it is the only method that gives the patient the possibility of getting a spontaneous pregnancy and restoring ovarian function, if extensive surgery is required due to an advanced endometriosis. It is important to note that the quality of oocytes and embryos can be affected in endometriosis, independent of the ovarian reserve, so the time of action is extremely important for a successful outcome.

CONCLUSION

The cumulative live birth rate (LBR) and pregnancy rate (PR) were significantly higher in the "freeze-all" versus fresh embryo transfer (LBR: 60.55% vs. 45%, PR: RR 1.30 [CI 95%]). The freeze-all strategy is associated with a lower obstetric risk [CI 95%]: the fetus has a lower risk of being small for gestational age RR: 0.59, low gestational weight RR: 0.74, premature birth RR: 0.74 but more increased risk of cesarean RR: 1.10 and large for gestational age RR: 1.49. With regard to the risks of antepartum haemorrhage, placenta praevia, perinatal mortality, congenital anomalies and spontaneous abortion rate, there are no consistent findings: some studies concluded that the risk is lower in freeze-all group (0.67, 0.68, 0.8 and, respectively 0,83), but most consider that there is no significant statistical difference. Gestational hypertension is controversial, some studies have found that it is more commonly linked to cryopreservation (RR: 1.29), but the results are insignificantly statistically different. However, cryopreservation is an independent risk factor for placenta accreta, 3 times higher than fresh embryo transfer. Monozygotic monochorionic twin pregnancy after single-embryo transfer is lower in freezed cycles in general (0.8%), but consider maternal age below 35 years a risk factor in the cryopreserved cycles.

All this information seems to make the "freeze-all" strategy an eligible protocol in the future.

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