STATE OF THE ART IN ASSISTED REPRODUCTIVE TECHNOLOGIES FOR PATIENTS WITH ADVANCED MATERNAL AGE

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Abstract

According to the WHO reproductive female age lasts up to 49 years, but problems with realization of women's reproductive rights may arise much earlier. A significant number of factors affect the state of reproductive health: socioeconomic; ecological; lifestyle features; the level of medical literacy; the state of the organization and medical care quality. Among the reasons for the fertility decline in advanced reproductive age are the loss of cellular receptors for gonadotropins; increasing the threshold of sensitivity of the hypothalamic pituitary system to the action of hormones and their metabolites and many others. Furthermore negative changes accumulate in the oocyte genome reducing the possibility of fertilization, normal development and implantation of the embryo and healthy offspring birth. Another theory of aging causing changes in oocytes is mitochondrial free radical theory of aging. Taking into account all these age-related changes in gametogenesis, this review considers modern technologies aimed at preserving and realization of female fertility. Among existing approaches two main ones can be distinguished: methods allowing to preserve reproductive cells at a younger age by ART intervention and cryobanking, as well as methods aimed at improving the basic functional state of advanced age women oocytes and embryos.

KEYWORDS: fertility preservation, advanced age women, oocyte, assisted reproductive technologies, platelet-rich plasma, mitochondrial donation.

INTRODUCTION

According to WHO reproductive health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity, in all matters relating to the reproductive system and to its functions and processes (World Health Organization WHO, 2021). Reproductive health implies that people are able to have a satisfying and safe sex life and that they have the capability to reproduce and the freedom to decide if, when and how often to do so. Despite the fact that according to WHO the reproductive age in females lasts up to 49 years (World Health Organization WHO, 2006), problems with the realization of their reproductive rights in women may arise much earlier. After 35 years, most women experience a decline in sexual functions and a deteriorating general health. Of course, this does not happen for everyone, but there is a high probability that a woman will face certain difficulties at this age. A slight decrease in reproductive function is noted from 27-28 years old, becoming even more pronounced at ages between 35 and 40 years old, and by the age of 45 reproductive function approaches zero.

Recently, women have increasingly realized their reproductive function at a later reproductive age when they have moved forward their career progression and acquired a certain material status necessary for full-fledged child care and upbringing. There are constant discussions about the expediency of motherhood in advanced reproductive age.

A significant number of factors affect reproductive health state: socioeconomic, ecological, lifestyle, the level of medical literacy, the state of the organization and the quality of their available medical care (Wu et al., 2000). They affect the womens' fertility in a different manner while age-related changes occur in everyone.

AGE - RELATED REASONS FOR FERTILITY DECLINE IN WOMAN

Negative changes accumulate in the oocyte’s genome with age. This reduces the fertilization possibility, normal embryo development should fertilization have been achieved, and implantation and healthy offspring birthrates (Franasiak et al., 2014). However, within the cohort of developing embryos from such aged women, many blastocysts with euploid chromosome set number are also unable to implant and develop into living embryos. Therefore age-related ooplasm abnormalities may also reduce oocytes’ competence even after fertilization and progress to adversely affect embryonic development (Capalbo et al., 2014).

Age determinate deterioration of morphological and functional oocyte characteristics and the embryos obtained from them have a multifactorial nature. It has been shown that the diameter of cytoplasm and thickness of *Zona pellucida* decrease linearly with the age of women (Valeri et al., 2011). Cell volume decreasing may be due to changes of ion pumps activity or the ability to regulate homeostatic water balance. Decreased oocytes quality may be associated with impaired lipid metabolism and decreased melatonin in follicular fluid (Cordeiro et al., 2011; M. Zhang et al., 2020).

Despite the contribution of the paternal genome to the embryo chromosomal balance, it is considered that the mother's age is the main cause of embryo aneuploidies. Age-related chromosomal abnormalities mainly arise because of meiotic impairments during oogenesis, following flawed chromosome segregation patterns such as non-disjunction, premature separation of sister or their reverse segregation (Cimadomo et al., 2018). The chromosomal aberration rate in MII stage oocytes of advanced reproductive age patients vary from 4% to 62% (Kuliev et al., 2005). Cytogenetic analysis of unfertilized oocytes showed an increase with age chromosomal abnormality rate (Petrushko, 2003). It is believed that the risks of aneuploidy may be associated with a long delay of the oocyte’s I stage meiosis which occurs before ovulation ovulation (Pellestor et al., 2003). It is known that oocytes telomeres begin to shorten due to the chronic effects of oxidative and genotoxic stresses over lifetime, the late exit of female gametes from the their cell cycle cessation as well as due to reduced telomerase activity (Keefe et al., 2006), cohesin dysfunction and meiotic spindle abnormalities (Keefe et al., 2015).

Evaluation of genes OCT4, SOX2, CDX2, GATA3, YAP expression and corresponding embryo proteins from young and advanced age women showed that the expression of SOX2 and CDX2 genes in embryos at the morula stage in the group of women over 38 years old was statistically lower than in the group of young patients (Gharanfoli et al., 2020).

The most common specific abnormalities in embryos of women older than 36 years are the sex chromosome monosomy (45, X) which accounts for almost 10% of all miscarriages and trisomies 16, 21 and 22, which together account for 50% of all miscarriages detected in spontaneous abortions (Gharanfoli et al., 2020).

Another theory of aging causing changes in the cell, including oocytes is mitochondrial free radical theory of aging (Barja, 2014). According to this theory, reactive oxygen species (ROS) and various toxic byproducts of aerobic metabolism, which are probably involved in cellular damage to genomes and mitochondrial DNA (mtDNA) and the associated with mutations. Increased production of ROS causes a clear disruption of the respiratory chain of the cell, leading to the accumulation of mutations and unbalanced redox activity (Taylor & Turnbull, 2005; Fragouli et al., 2015). Basal ROS levels often decline during the normal mitochondrial function phase within the respiratory chain, while the excess amount of cellular ROS is determined by aging, which contributes to a significant increased oxidative damage and loss of cellular activity. Thus, there is a change in mitochondrial function: decreasing of mitochondrial membrane potential (MMP), oxidative phosphorylation (OXPHOS) and the number of mtDNA copies in advanced age women comparing with younger women (May-Panloup et al., 2016). This coincides with lower mtDNA levels in advanced age woman embryos at the early cleavage stage, but paradoxically the opposite was observed at the blastocyst stage, with higher mtDNA levels associated with increased aneuploidy and failed implantation (Fragouli & Wells, 2015; Liu et al., 2017).

ART FEATURES FOR ADVANCED REPRODUCTIVE AGE WOMEN

An increase in serum progesterone and estradiol levels is observed in advanced reproductive age patients (Chahal & Drake, 2007). They have a poor response to stimulation of superovulation (Seifer et al., 2011). The oocytes fertilization rate is significantly lower in patients aged more than 36 years comparing with younger ones (Asada et al., 2019). All of these factors lead to the fact that the effectiveness of ART programs in women over 40 years old is about 5% (J. Zhang, 2015). These unsuccessful ART outcomes pushed reproductive specialists and scientists to carry out scientific research to improve reproductive indicators for advanced age women. Among existing approaches two main ones can be distinguished: methods allowing to preserve reproductive cells at a younger age by ART intervention and cryobanking, as well as methods aimed at improving the existing functional indicators of oocytes, embryos of advanced age women to aid optimal selection during ART.

CRYOTECHNOLOGY AS THE BASIS FOR PRESERVING REPRODUCTIVE POTENTIAL OF ADVANCED AGE WOMEN

Economically developed countries pass laws on reproductive health protection. Scientists are developing methods to prevent reproductive health and prolong it for the longest possible period. Modern methods of preserving reproductive health and prolonging the reproductive period of women are based on the use of cryotechnology.

Oocytes’ freezing for so-called "social reasons" female groups applies to women who seek to prolong, protect and secure their fertility for later years (Simopoulou et al., 2018). In 2013, the American Society for Reproductive Medicine for the first time approved cryopreservation of oocytes as non-experimental (Society for Assisted Reproductive Technology, 2013). Experience has shown that cryopreservation of oocytes is a promising area for maintaining female fertility. However, it is important not only to obtain a high survival rate of oocytes after cryopreservation and preserve their main function, but also to ensure the absence of negative effects of cryopreservation factors on the genome of embryos that developed from oocytes after long-term low temperature storage.

Data on the chromosomal status of embryos derived from cryopreserved oocytes are quite contradictory. Some studies have found that cryopreservation factors can induce meiotic spindle abnormalities and chromosome disorganization in human oocytes, which, in turn, may increase appearance of embryos with aneuploid chromosome sets number (Bromfield et al., 2009). However we have previously shown that the level of chromosomal anueploidy of embryos derived from cryopreserved and fresh oocytes did not exhibit significant differences (Buderatska et al., 2020). It should be noted that such results were obtained in a study of women with a mean age of 27.6 ± 4.8 years.

There are very few data in the scientific literature on autologous oocytes cryopreservation for advanced age women with successful pregnancy and childbirth (Parmegiani et al., 2009).

Recent publications reported that survival rate after cryopreservation was increased recent years, which confirms the influence of methodological approaches to female gamete cryopreservation provided by optimal ART (Pai et al., 2021). The turning point in the improvement of oocyte cryopreservation methods was the use of vitrification (Kuleshova et al., 1999). The absence of ice crystals formation when the temperature was lowered to -196 C and at the stage of warming made it possible to reduce the level of cell damage and significantly increase the efficiency of oocyte cryopreservation comparing with slow freezing (Levi-Setti et al., 2016). Also using different technique prior to cryopreservation of oocytes and embryos at various preimplantation stages could help to preserve their functional characteristics in the women of younger ages (Petrushko et al., 2018; Petrushko et al., 2019). It should be noted that the state of the endometrium is also very important, when transferring embryos which contributes to the level of implantation. Women older than 38 years have reduced implantation (6.5% vs. 10.9%, p = 0.01) and pregnancy (10.1 vs. 18.7%, p = 0.02) rates compared to younger women (Borini et al., 2010). More optimistic results of the incidence of pregnancy are given in the work of L. Rienzi - 12.6% for women in the older group against 27.5% for women younger than 38 years (Rienzi et al., 2012).

The results of oocyte cryopreservation depending on woman age revealed that "take home baby" rate ranged from 5% to 17% in patients older than 40 years. The birth rate in patients aged 41–42 years was 2% –3% lower than in patients aged 37–40 years (Cil and Seli, 2013).

Data from the US Reproductive Technology Association in 2013 showed that live births rate in cycles with fresh embryos from non-donor oocytes decreases from 21% at the age of 38-40 to 11% at the age of 41-42 with further reduction to 4.5% for age under 42 years.

Cryopreservation of oocytes by social indicators in younger age and subsequent *in vitro* fertilization and embryo transfer gives women who expect to become pregnant in advanced age two important benefits: becoming genetic parents to their own offspring and reducing the risk of having children with chromosomal abnormalities associated with aneuploidy. In addition, freezing oocytes may be the best alternative for women who do not have a partner at a particular stage in life or who have moral prejudices about embryos cryopreservation (Buderatska and Petrushko, 2016).

The use of cryotechnology to preserve women’s fertility in young age is not limited to cryopreservation of oocytes. This is due to the fact that in contrast to the “social reasons” for postponing of conception, there are also medicinal ones, in which there are a number of contraindications for induction of superovulation in order to obtain mature oocytes. In such cases, ovarian tissue cryopreservation (OTC) is recommended before gonadotoxic therapy for the purpose of further transplantation after recovery at an older age (Kim et al., 2018). OTC also can be offered as an alternative to preserve fertility in young patients at risk of premature ovarian insufficiency. However, despite significant advances in the development of methods for OTC, a little more than 130 children have been born to date. Restore fertility using OTC could be realized by ovarian tissue transplantation or *in vitro* maturation with followed by *in vitro* fertilization. It should be noted that transplantation of ovarian tissue has a number of contraindications, especially in cases where the ovary is involved in the malignancy. The efficiency of OTC procedure is questionable above 36 years of age and it is still considered as experimental in many countries, and legislations and regulations vary (ESHRE, 2020).

TECHNIQUES IMPROVING OOCYTE AND EMBRYO QUALITY OF ADVANCED AGE WOMAN

Methods for improving of fresh or cryopreserved oocytes and embryos quality are proposed: for example, co-culture systems with Vero, oviduct and endometrium cells (Moshkdanian et al., 2011); microsurgical removal of fragmentation in day 2 embryos (Kim et al., 2018), individualization of the embryo day transfer etc. (Yoon et al., 2001). The most promising method of oocyte renewing taking in to consideration mitochondrial free radical theory of aging is mitochondrial replacement therapy (MRT). As known that, unlike the nuclear genome, which is inherited equally from both parents, the mitochondrial genome is transmitted mainly from the mothers’ gamete (Zou et al., 2020). Mitochondrial DNA is circular in structure and contains 37 genes controlling production of proteins involved in energy metabolism. Thus mtDNA mutation can affect organs that depend on it, giving rise to several incurable diseases, such as: deafness, diabetes mellitus, myopathies, glaucoma and others (Costa et al., 2016). MRT could be performed by several techniques each of them has advantages and disadvantages.

OOPLASM TRANSFER

The first donor cytoplasm containing healthy mitochondria along with sperm was transferred to the 39-year-old recipient oocyte's cytoplasm using ICSI procedurein 1997 (Cohen et al., 1997). In this case, the reconstituted zygote contains the original parent nuclear DNA and mixed mtDNA from both donor and recipient oocytes. However, this technology was later banned due to mitochondrial heteroplasm with two types of mtDNA and chromosomal abnormalities in the future offspring (Barritt et al., 2001; Mobarak et al., 2019).

MEIOTIC SPINDLE TRANSFER

The first live birth case in human using meiotic spindle transfer was reported by Zhang et al. in a 36-year-old woman diagnosed with Leigh syndrome (Zhang, Liu et al., 2016). The metaphase II meiotic spindle was extracted from the recipient's oocyte and transferred to a healthy donor oocyte with a pre-removed own spindle. After fertilization of the reconstructed oocyte by ICSI the resulting zygote contains mitochondria from a healthy donor and the original nuclear DNA of the parents. Using this method of MRT less than 1% of maternally affected mtDNA has been transferred to offspring (Craven et al., 2017). However, this indicator of the maternal mtDNA content may fluctuate depending on technical aspects such as competency of the embryologist involved.

POLAR BODY TRANSFER (PBT)

To perform PBT, the first polar body of the recipient's oocyte is transferred to the enucleated donor's oocyte. After that, the reconstructed oocyte is fertilized by the ICSI method. Besides the first PBT, it is possible to carry out the transfer of the second PB. After fertilization of the recipient's oocyte secretes the second polar body which is extracted and transferred to the donor's zygote with the previously removed female pronucleus. PBT has several advantages such as minimum transfer of affected patients mtDNA due to the fact that PB includes few mtDNA copies due and PBT yields undetectable carryover of donor mtDNA in two generations (Wang et al., 2014). Also PBT leads to less chromosomal damage due to the PB structural features and location. Moreover in case of the first PBT this technique could be used to double the number of oocytes in the case of poor responders with the absence of mtDNA abnormality.

PRONUCLEAR TRANSFER (PNT)

This technology of mitochondrial transfer consists in the fact that pronuclei of one zygote are transferred to another enucleated zygote. To do this, a donor oocyte and an oocyte with a disturbed mitochondrial DNA are fertilized with the sperm of the future father. Shortly after the appearance of pronuclei, a pronuclear transfer is carried out into the zygote obtained from the donor oocyte. Thus, the embryo carries the nuclear DNA of the parents, and the donor mitochondrial DNA.

This technique has number of advantages, since pronuclei are well visualized under a light microscope without the use of additional equipment, and the fertilization procedure can be performed by ICSI as well as IVF. However, from an ethical point of view, the implementation of this technique requires the destruction of the embryo at the zygote stage, and it is this method of mitochondrial transport that has the greatest limitations in some efforts (Ishii and Hibino, 2018). In addition, for the fusion of pronuclei and donor cytoplasm, the inactivated viral vector SeV (Sendai virus, also known as HVJ-E) is used (Tachibana et al., 2009; Craven et al., 2010). The absence of viral genetic material has been shown (Tachibana et al., 2009).

The first report of a successful pregnancy with fetus bearing a normal karyotype and low heteroplasiarate was received in 2016 in a 30 year old woman with two unsuccessful IVF attempts, as a result of which all the resulting embryos stopped developing at the two-cell stage (Zhang, Zhuang et al., 2016).

GERMINAL VESICLE TRANSFER (GVT)

Immature oocytes in prophase I of the first meiosis have a nucleus which stays in form of “germinal vesicle” until meiosis resumption. GVT is removed and transferred to a donor oocyte with previously extruded its germinal vesicle. The ooplast and karyoplast merger is conducted by electrofusion with an electro cell manipulator then the reconstructed oocyte undergoes *in vitro* maturation followed by fertilization.

This technique was firstly applied for advanced reproductive age women by Zhang et al in 1999, however, the reconstructed oocytes have not reached the mature stage (J. Zhang et al., 1999).

The advantage of GVT is that it can be performed before the onset of MI meiosis. Since mitochondria play an important role in chromosome segregation the restoration of mitochondria prior to meiosis I may contribute to a higher level of euploid set of oocyte chromosomes and the development of a normal embryo after fertilization (Tanaka and Watanabe, 2018). Whether GVT can save chromosomal abnormalities in age-related oocytes needs further study.

AUGMENT TECHNOLOGY

Autologous germline mitochondrial energy transfer (AUGMENT) is the strategy launched by OvaScience in 2014 to transfer producing energy mitochondria from autologous egg precursor cells into the oocytes. This action has been shown to enhance the quality of the egg and fetus (Fakih, 2015; Cozzolino et al., 2019). The results of other researchers indicate the lack of a positive effect on fertilization, the development of euploid embryos for patients whose average age was 36.3 ± 3.6 years, and further studies of the effectiveness of this method on oocytes of older women which are characterized by a pronounced decrease in energy reserves are required (Labarta et al., 2019).

Autologous mitochondrial injections are also carried out with the use of cumulus and granulose cells which improve the fertilization rate, the quality of the embryos for 3 days and the pregnancy rate (Tian et al., 2019). However, the use of autologous mitochondrial injections is useless in situations of mitochondrial mutations, which can increase with age.

STEM CELLS FOR GERM CELL PRODUCTION

Another alternative direction for reproduction of advanced age woman is reconstituting gametogenesis *in vitro*. In the renewal of ovarian reserve and the competence of germ cells is the production of mature gametes from germ cell progenitor stem cells, embryonic stem cells, tail tip of fibroblasts, or even by reprogramming granulosa cells into oocytes (Tian et al., 2019; Hikabe et al. 2016; Hayashi et al., 2012). Successful *in vitro* reconstitution of primordial germ cells has recently had a significant effect in the field. The authors have demonstrated entire process of mammalian oogenesis in mice *in vitro* from primordial germ cells, birth of fertile offspring obtained from a cultured gonad (Morohaku et al. 2016).

Skin derived stem cells (SDSCs) constitute a heterogeneous population of stem cells generated *in vitro* from dermis, which can be cultured as spherical aggregates of cells in suspension culture. Under certain *in vitro* or *in vivo* conditions, SDSCs show multipotency and can generate a variety cell types (Ge et al., 2016). It has been shown that SDSCs are able to produce primordial germ cell-like cells *in vitro*, and even oocyte-like cells. Whether these germ cell-like cells can give rise to viable progeny remains, however, unknown.

USING OF PRP THERAPY FOR FERTILITY PRESERVATION OF ADVANCED AGE WOMEN

Ovarian tissue rejuvenation due to the paracrine effect of platelet-rich plasma (PRP) has become a fairly new and actively developing direction (Cakiroglu et al., 2020).

Many studies have documented that the use of PRP can reduce the signs of inflammation, postoperative blood loss, infection, and in addition leads to accelerated osteogenesis and healing of wounds and soft tissues (Park et al., 2011). Many articles report that PRP therapy implementing can enhance healing and the anti-aging process, employing angiogenesis regeneration due to the multiple growth factors and cytokines involved (Du et al., 2020).

PRP realizes its effects due to cytokines and growth factors that are contained in platelet granules, such as transforming growth factor-β, insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), granulocyte colony-stimulating factor (G-CSF) and many others (Amable et al., 2013; Ramaswamy et al., 2018).

The addition of Ca^{2+} to PRP leads to the release of platelet granule contents and activation of processes induced by growth factors (Amable et al., 2013). PRP cryopreservation can activate platelet granules in addition to the advantage of a prolonged shelf life (Kleinveld et al., 2020; Kelly et al., 2019).

The use of PRP therapy could be applied in the fight against infertility of women with a poor response, premature ovarian insufficiency and late reproductive age, who do not want to carry out the oocyte donation program, made it possible to improve the hormonal background and activate folliculogenesis in the ovaries (Melo et al., 2020; Sills et al., 2020).

Given the inverse relationship between aging, growth hormone concentration and IGF-1 (Fanciulli et al., 2009), the use of PRP reached in various cytokines and growth factors, including IGF-1, for injections into ovarian tissue leads to an increase in cell proliferation and the follicles development.

It was shown that the injection of autologous PRP into the ovarian tissue of women with limited ovarian reserve or absence of menstruation for more than 1 year improved fertility in all patients aged 42 ± 4 years. Thus, 5.3 ± 1.3 MII oocytes and at least one embryo suitable for freezing were received for each patient in IVF cycle in 78 days after treatment (Sills et al., 2018). Similar results were obtained by other authors, who reported an improvement of hormonal status and pregnancy rate including natural, after PRP therapy in women with low ovarian reserve (Petryk et al., 2020). Since 2019, several cases of childbirth after PRP therapy have been published as well as the onset of natural pregnancy in women with premature menopause (Farimani et al., 2019; Pantos et al., 2019).

Except injection into the ovarian tissue PRP could be used to increase the effectiveness of oocyte *in vitro* maturation methods, which is relevant for patients with polycystic ovary syndrome, poor responders, fertility preservation for women of different reproductive ages (Yurchuk et al., 2021). It has been shown that addition PRP into the maturation media increase the quality of bovine mature oocytes (gametes with a high mitochondrial potential), and leads to an improvement in bovine embryos development (Moulavi et al., 2020; Ramos-Deus et al., 2020) Thus, despite encouraging achievements of *in vivo* and *in vitro* PRP using for improving fertility of patients with advanced maternal age, more extensive data base evidence are requested.

The advantages and disadvantages of the main methods for improving oocyte function and fertility potential are reflected in Table.

CONCLUSIONS

The possibility of realization of reproductive function in women of late reproductive period is one of the urgent problems of modern science. Assisted reproductive technologies have advanced in the fight not only with infertility in young women, but also in older patients. Some approaches are aimed at improving the reproductive function of women of late reproductive age by preserving fertility at an early age namely by cryopreservation of oocytes or embryos. Physicians should inform all patients of reproductive age about reduced fertility after the age of 35 and recommend not postponing pregnancy planning to late reproductive age or preserve fertility using cryopreservation techniques. Patients should be informed about the current possibilities of science to ensure the preservation of reproductive function through the use of cryotechnology and to allow them to make informed choices.

Other approaches are aimed at improving the quality of oocytes and embryos obtained from women of late reproductive age. Among these technologies, the methods of mitochondrial replacement are especially prominent, as the main source of aging of female oocytes. Such technologies are aimed at preserving the mother's nuclear genotype to make possible generating genetically one's own child in advanced reproductive age patients. Whilst these technologies are pushing the boundaries for manipulating female reproductive cells to achieve better outcomes in ART, the optimistic results of studies on gametogenesis reconstitution from somatic stem cells do not yet make it possible to implement them in clinical practice and require significant further research and validation. At the same time, the growing popularity of the use of PRP therapy to improve reproductive hormonal background and activate folliculogenesis in women of older reproductive age requires further in-depth research and has good prospects for treatment.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

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Table 1

Method	**Advantages**	**Disadvantages**
Oocyte cryopreservation	High survival, fertilization, embryo development and pregnancy rate	Oocytes of advanced reproductive age have lower quality and may have additional damage during cryopreservation
Embryo cryopreservation	High survival, embryo development and pregnancy rate	Ethical point, in case of the absence of a permanent partner

Ovarian tissue cryopreservation	Recommended before gonadotoxic therapy	Age above 36 years old, the risk of malignant cell reinroduction in some cases, not very high birth rate after trasplantation.
Ooplasm transfer	Improving mitochondrial oocyte status	Mitochondrial heteroplasm with two types of mtDNA
Meiotic spindle transfer	Reconstructed oocyte by ICSI the resulting zygote contains mitochondria from a healthy donor and the original nuclear DNA of the parents. Less than 1% of marternal affected mt DNA	The maternal mtDNA content may fluctuate depending on the performer qualifications
Polar body transfer	Minimum transfer of affected patients mtDNA less chromosomal damage could be used to double the number of oocytes	-
Pronuclear transfer	Ppronuclei are well visualized that makes easier to perform the procedure, fertilization procedure can be conducted as ICSI and IVF	Ethical point deals with embryo distraction
Germinal vesicle transfer	Could provide positive effect on chromosome segregation if performed before the onset of MI meiosis	Not well developed *in vitro* maturation technique
AUGMENT technology	Enhance the quality of the egg and fetus	Mitochondrial heteroplasm, useless in mitochondrial mutations
Stem cells	Entire process of mammalian oogenesis in mice *in vitro* have been demonstrated	Still developing technique
PRP therapy	Improve the hormonal background and activate folliculogenesis in the ovaries	Need more knowlage in this area