Review Article
Open Access

Unexplained infertility: a fresh look at the old problem and the novel therapeutic options of its treatment

Abstract

Infertility affects up to 20% of couples worldwide. Among the frequent causes of female infertility are fallopian tubes-related disorders, hormonal and ovulation disorders, endometriosis, and unexplained infertility. The modern-day tendency to delay pregnancy has increased the incidence of age-related infertility, as female reproductive competence decreases with aging. Aging is associated with low-grade inflammation, mitochondrial dysfunction, reduced capacity of antioxidant protection system, and stem cell exhaustion in female reproductive system. Hence, the appropriate actions should be made to address the infertility caused by reproductive aging, oxidative stress, and mitochondrial dysfunction. In recent years, a considerable progress in cell therapy as an emerging approach for the treatment infertility has been made. Cell therapy involves utilizing stem cells, precursor cells, cellular extracts, exosomes and other cell-derived therapeutic agents. Cell therapy can be an effective strategy as it provides an interactive, dynamic, specific and individualized treatment.

Keywords: infertility, cell therapy, stem cells, reproductive function, ovaries, hormonal disorders, mitochondrial dysfunction, oxidative stress, regenerative medicine

Volume 15 Issue 1 - 2024

Margarita Yemeliyanova,[1] Mike KS Chan,[1,3] Michelle BF Wong,[1,3] Dmytro Klokol[1,2]
[1]European Wellness Biomedical group (EU,APEC), Germany
[2]European Wellness Academy, Malaysia
[3]FCTI Research & Development GmbH, Germany

Correspondence: Margarita Yemeliyanova, MD, European Wellness Biomedical group (EU,APEC), Germany, Email dr.margaryta@sbi-europe.com

Received: January 11, 2024 | **Published:** January 22, 2024

Introduction

Infertility is known as an inability to conceive (regardless of cause) after one year of consistent (at least two times a week) unprotected intercourse when a female partner is younger than thirty-five years old or within six months if older than thirty-five years of age. The occurrence of infertility is around 14% of the general population, thus affecting roughly 1-in-7 couples. Among the most known causes of infertility are ovulatory disorders, male factors, fallopian tube pathology, uterine and/or peritoneal conditions.[1]

As a rule, the diagnosis of unexplained infertility is established via ruling out the other causes of infertility after using standard fertility tests, including spermogram, ovulation tests, and tubal patency testing. It accounts for almost 40% of cases of female infertility, and involves from 8% to 28% of couples, experiencing fertility problems.[2]

The establishment of an unexplained infertility diagnosis is based on the investigations done prior to diagnosis. Some of the affected couples who are investigated for the unexplained infertility may successively conceive spontaneously. Their rate of spontaneous successful pregnancy reaches 2-4%. The most significant prognostic factor for successful spontaneous conception is woman's age, which has a higher conception rates below 30 years. After one year of unsuccessful attempts, half of females with unexplained infertility may conceive during the subsequent year, and 12% more of the females may conceive within the next two years.[3] But women nowadays cannot wait too long since their childbirth plans have already been delayed significantly due to cultural and social changes.

Oxidative stress and mitochondrial dysfunction in female reproductive system

A disproportion in the amount of naturally-occurring antioxidants and reactive oxygen species (ROS), with the accumulation of the ROS, creates perfect conditions for oxidative stress. Oxidative stress causes lipid peroxidation, protein peroxidation, and genomic damages to DNA and RNA.[4,5] The oxidation of phospholipids disturbs the integrity of cellular membranes. The peroxidation of RNA and DNA causes its degradation, which can be a trigger to programmed death of the cells.[5–7] Impaired adaptation mechanisms against the oxidative stress causes mitochondrial dysfunction within the cell by inactivating the enzymes of the mitochondrial electron transport chain and by promoting the mutations in mitochondrial DNA. Apart from that oxidative stress has also been linked to telomere shortening and cellular senescence.[8] Mitochondrial dysfunction diminishes ATP production and has a negative impact on the antioxidant synthesis. Such situation creates a vicious cycle when mitochondrial dysfunction caused by the free radicals further increased production of ROS and worsen mitochondrial damage.[9]

Oxidative stress and mitochondrial dysfunction are initiated by both endogenous and exogenous factors. The endogenous factors are biological age, endometrial disorders, polycystic ovarian syndrome (PCOS), and premature ovarian insufficiency (POI).[10] While exogenous factors comprise of environmental exposure to the inducers of ROS - diet, occupational hazards, and assisted reproduction treatment techniques.[11]

Cellular and subcellular aging mechanisms contributing to infertility

Aging process implicates not only the deterioration of the physiological functions of the organism, but also impairs the fertility of the aging individual. The advanced maternal age is associated with the increased risk of adverse obstetric outcomes such as miscarriage, preeclampsia and eclampsia, increased occurrence of pre-term and/or post-term delivery, low birth weight and neonates that are small or large for their gestational age, and C-section.[12,13] Hence, the constantly increasing occurrence of female infertility and consequent need in assisted reproductive technologies.

According to the research of the last few decades, the failures to conceive and low pregnancy rates performed both naturally or with

the help of assisted reproduction technologies can be related to low-quality oocytes and sperm cells. It has also been proven that the quality of gametes directly depends on the number of mitochondria they contain and the mitochondrial function. Low gametes quality can also be the reason for a decline in response to ovarian stimulation, reduced embryo quality and pregnancy rates, as well as an increased incidence of miscarriages and fetal aneuploidy. Couples 35 years and above with unexplained infertility are the most representative category of patients who are implicated to have low gamete quality due to aging of their reproductive system.

The aging process itself and any age-related pathologies nowadays are largely associated with the mitochondrial malfunction, mostly due to the accumulation of multiple deletions and mutations in the mitochondrial DNA (mtDNA) stran.[14] Among other genetic defects observed in aging oocytes are reduction of intracellular antioxidants,[15] and acceleration of apoptosis in oocytes,[16] abnormal calcium oscillation signals,[17] and high incidence of aneuploidy.[18]

Mitochondrial dysfunction in female reproductive disorders

Embryonic oocytes are usually formed at the fetal development stage and are present inside the ovary for nearly 50 years before they grow and develop into matured oocytes. The ovulation process leads to a continuation of meiosis in the immature oocyte, making it mature and ready for conception. This implies the process of chromosome alignment and separation by the nuclear spindle, leading to the reduction of chromosomes to the amount of 23, where another 23 chromosomes appear to be isolated outside of the oolema and enclosed subsequently in the first polar body. Once penetrated by a healthy spermatozoa, what happens next is the extrusion by the oocyte of 23 sister chromatids enclosed in the second polar body. That is how a fertilized zygote receives a normal diploid set of 46 chromosomes. The process of chromosome extrusion outside the oocyte and forming the first and second polar bodies is quite energy-consuming, and it appears to be supplied by mitochondria. The oocyte cell encompasses the largest number of mitochondria and mitochondrial DNA (mtDNA) copies compared to muscle cells and neurons, which have higher energy requirements and contain thousands of mtDNA copies. Upon the follicle recruitment process, the number of mitochondria in the oocyte cell is markedly increasing, from 6,000 mtDNA copies to 200,000, comprising nearly 50% of the total DNA content in the oocyte.[19,20]

The structure of mtDNA differs from that of nuclear DNA. It is also a double-stranded circular shape, containing 16,569 pairs, though it has no histones or introns like nuclear DNA, which makes it more susceptible to mutations and deletions. The mtDNA includes 37 genes encoding proteins taking part in the respiratory chain of ATP production. The embryo cells always inherit maternal mtDNA, as the paternal one undergoes degradation and elimination via ubiquitination reactions.[21]

The aging process is well-known to be accompanied by mitochondrial dysfunction and decreased energy production, which results in impaired oocyte maturation, so the very important process, such as nuclear spindle activity or chromosomal segregation, appears to be seriously deteriorated. With age, the mitochondrial energy production in oocytes depletes, and processes like oocyte maturation with its nuclear spindle activity and segregation of the chromosomes deteriorate.[22]

As a result, the aneuploidy rate increases, especially the trisomy, frequently observed in older women's offspring. There is data that the number of mutations in mitochondria of the follicle cells increases with age, resulting in impaired reactions of ATP production and oxidative phosphorylation in older women.[22] It was shown that chances for successful implantation strongly correlate with the ATP production and content in embryo cells. The study demonstrated that a reduced capacity of oocytes to produce ATP molecules leads to an abnormal nuclear spindle and random chromosome spreading.[22,23]

Aging of human oocytes is closely associated with shifts in mitochondrial function, mitochondrial DNA copies numbers, and mitochondrial mutations.[24,25] Simultaneously, in aging oocytes the significant increase in ROS levels and upregulation of mtDNA stress genes is observed. Notably, the hallmark of the aging oocyte are lower mtDNA and a number of essential mitochondrial dysfunction.[26] Research data on age-related changes in mtDNA in human oocytes suggests both the decrease,[27] as well as increase of mtDNA.[28] In a study of Fragouli the mtDNA copy number was decreased in cleavage stage embryos in females of a reproductively older age (average age 40 years), and increased in blastocysts in comparison to females in a younger reproductive age (average age 35 years).[29] Experimental studies reveal that mitochondria from old mice oocytes are different compared to young mice' oocytes, and matured oocytes from old mice have considerably lower amount of mtDNA than young mice. The size and the total area covered by mitochondria in an oocyte vs total area of the oocyte's cytosol was smaller in oocytes of older mice. Oocytes of the elder mice also have a less mitochondrial density compared to young ones. In general, the differences in mitochondrial status of young and old oocytes show the reduction in the functional capacity of mitochondria associated with ageing.[26]

The aging oocytes have their telomerase activity also decreased, which, in its turn, could further contribute to the chromosomal damage in oocytes.[30] The relationship between oxidative stress and ovarian aging is closely linked to abnormal mitochondrial functions, accumulated mutations and the downregulation of mitochondrial antioxidant gene expression in aged oocytes.[31] In fact, this shifts not only concern the mitochondria, but it is also well established that oxidative stress in main biological molecules (proteins and lipids), as well as in DNA of the aged oocytes causes the decline of the quality of the oocytes.[32]

In the ovaries, under normal circumstances the ROS generated during an inflammatory reaction from immune cells and cytokines in the follicular fluid induce oocyte maturation and subsequent follicle rupture and ovulation.[33] Hence, the ROS produced at physiological level by the follicles are necessary for ovulation. On the contrary, inhibition of ROS would suppress the ovulation.[34] Conversely, the excess of the ROS resulting in oxidative stress triggers abnormalities in female reproductive system, representing premature ovarian failure (POF), POI, and PCOS. POF, which usually means early menopause (before the age of 40 yo) is associated with prematurely impaired ovarian function due to abnormal development or depletion of follicles due to increased apoptosis.[35] On the other hand, a drop in levels of endogenous antioxidants and increased oxidative stress in patients with PCOS produces abnormal formation of cysts and ovarian tissue remodelling, which leads to absence of ovulation and subsequent infertility.[36]

Albeit that ROS are generated in the corpus luteum post-ovulation playing a major role in progesterone synthesis, which is essential for the development of the uterine environment and in regulation of implantation, survival, and the progression of pregnancy, the excessive production of free radicals and oxidative stress to the corpus luteum disturbs the progesterone synthesis, which can be detrimental to embryo and development of pregnancy.[37]

In the uterus, the excessive oxidative stress disturbs morphology and function of the uterus, including detachment of the endometrial epithelium and possible hindrance of implantation.[38] During pregnancy, oxidative stress can lead immune dysfunction in the uterus that may lead to an early pregnancy loss.[39] The impaired anti-oxidative stress protection mechanisms interfere with the successful implantation.[40,41]

Oxidative stress and energy production-utilization pathways affect not only the oocytes, ovaries and uterus, but also the embryo. As a rapidly developing organism with high energy demand, the embryo gets the energy supplied by ATP produced via mitochondrial oxidative phosphorylation and glycolysis. The studies on mice demonstrated that post-compaction embryos consume times more oxygen than those in earlier stages of development and tend to shift to glucose utilization metabolic pathways.[42] Because the mitochondria in embryo cells do not replicate until the blastocyst stage, its pool must be divided between all the numbers of increasing cells during the embryo cleavage. So, its metabolic activity must also increase according to the expanded cellular activity. Hence, we can suggest that the correlation between older maternal age and the risk of chromosomal abnormalities occurring in offspring is reasonably attributed to depleted mitochondrial activity in the oocytes, which leads to both non-disjunction of the chromosomes and arrest of embryo development.

Therapeutic modalities in unexplained infertility in Bioregenerative medicine

In this review, we emphasize that mitochondrial dysfunction plays a significant role in reproductive failures and propose that reproductive function in women can be improved largely with the use of mitochondrial peptides and other mitochondrial nutrients extracted from the xenogeneic stem cells derived from various fetal tissue - ovaries, testis, and placenta at a first place, as well as from other organs belonging to hypothalamus-pituitary-adrenal-gonadal (HPAG) axis.

Despite significant advancements in assisted reproduction techniques, such as the intrauterine insemination and the in vitro fertilization (IVF), these techniques do not recreate the ideal conditions of natural impregnation. The presence of underlying mitochondrial dysfunction and failure of anti-oxidative stress defence mechanisms in reproductive system, require the supplementation of the human IVF culture media with the biologically active molecules capable of modulating the mitochondrial function, stimulators of mitochondrial genesis and biologically active substances with antioxidant properties. Such molecules include naturally-occurring peptides of HPAG axis, coenzyme-Q10, folic acid, vitamins A, C, and E, pantothenic acid, melatonin, resveratrol and others.[43,44]

Thus, resveratrol exhibits therapeutic effects in treatment of many diseases due to its anti-aging, antioxidant, anti-inflammatory, insulin-upregulating effects, cardioprotective, and anti-neoplastic properties.[45] Resveratrol may be beneficial for the women with impaired ovarian function, PCOS, endometriosis, and uterine fibroids.[46,47] The beneficial effects of resveratrol are exerted through the sirtuin 1 (SIRT1) activation.[48] Another pathway through which resveratrol inhibits oxidative stress and inflammation in POI model and exerts its anti-apoptotic effects, hence improving the ovarian dysfunction caused by POI, is through the inhibition of the PI3K/AKT and the NF-kB signalling pathways.[49–51] In addition, resveratrol inhibits theca-interstitial cell androgen production. Therefore, resveratrol is found beneficial in treatment of PCOS - a condition closely associated with insulin resistance and hyperinsulinemia, theca-interstitial cell hyperplasia, and hyperandrogenism.[52] In the endometrium, resveratrol exhibits anti-apoptotic and anti-proliferative effects. Moreover, resveratrol reduces expression of the vascular endothelial growth factor (VEGF), thus aiding in management of endometriosis and ovarian hyperstimulation syndrome, as both of these conditions are related to the excessive VEGF activity.[53]

Coenzyme-Q10 (CoQ10) is the carrier-transporter of electrons in the mitochondrial respiratory chain between complexes I, II, and III. Thus, CoQ10 participates in the synthesis of ATP.[54] Being a source for superoxide anion, CoQ10 acts both as a prooxidant as well as an antioxidant. Its reduced form - the ubiquinol, a potent antioxidant, protects biological membranes from lipid peroxidation.[55] Addition of CoQ10 to the treatment protocols improves mitochondrial function, and through that pathway may improve the outcome in infertile patients. CoQ10 treatment prevented mitochondrial ovarian aging, and restored the age-related decline of oocyte quality.[56]

Lastly, CoQ10 administered to the aged mice restored mitochondrial respiratory function and increased glucose uptake in cumulus cells, hence helping to improve the reproductive function. The human studies shown that higher levels of CoQ10 in follicles are linked to a better embryo quality and higher pregnancy rates.[57] CoQ10 supplementation (600 mg/day for 2 months before ovarian stimulation) increased ovarian response, fertilization rates, and the number of high-quality embryos in young women with poor ovarian reserve.[58] The pre-treatment before IVF with CoQ10 increases successful pregnancy rates.[59]

Folate, vitamin B9, and its synthetic form called folic acid, is common in dietary supplements due to its high bioavailability and massive health benefits.[60] The main biochemical function of folate on the cellular level is the donation of the methyl group for the homocysteine to convert into methionine via methylation reaction,[61] which is a crucial transitional compound in endogenous synthesis of glutathione - the potent intracellular antioxidant. Consequently, folic acid exerts protection from oxidative stress by increasing endogenous expression of antioxidant in the cell. Hence, folic acid supplementation is widely used in the reproductive field, as well as during pregnancy and is essential in achieving favourable pregnancy outcomes.[60,61]

Cell therapy as a novel therapeutic modality in unexplained infertility

In recent decade we are gathering more and more evidence that cell therapies can provide novel therapeutic paradigms in reversal of a wide range of degenerative and age-related disorders.[62–64] New developments in molecular biology and cellular research have greatly expanded the clinical indications of these therapeutic modalities to unexplained infertility as well.[64] The rationale behind this is that majority of disorders leading to the infertility and other reproductive disorders do not appear due to the deficiency in one protein or enzyme, but the changes in the complicated signalling on cellular and subcellular level. Cell-based therapy is an efficient modality that provides an interactive, dynamic, and individualized treatment, which addresses to patient's pathophysiological conditions.

Uterine tissue immune microenvironment has a crucial role in maintenance of pregnancy. The immunogenic cells, such as Placenta-derived stem cells, Thymic progenitor cells and bone marrow-derived cells and their cytokines act as the key regulators. These cells express numerous cytokines, such as interleukins IL- 1α and IL-1β, TNF-α and exert positive affects on the endometrium. It also promotes the invasion and hemochorial placentation and regulates immune status of the embryo during implantation.[65] Thus, Yoshioka in 2006 has

reported the direct effects of cell therapy on the human endometrium. The study concluded that the intrauterine administration of the stem cells, co-cultured in media with added HCG, has notably enhanced implantation and increased the live birth rates in cases of recurrent IVF failure.[65]

According to Hashii et al. stem cells co-culture with luteal cells derived from pregnant cases enhanced the Th2 cells cytokines production (IL-4 and IL-10), induced endometrial differentiation and promoted embryo implantation.[66] Th1 cells enhance cytotoxicity function of NK cells via secretion of cytokines with pro-inflammatory properties (IL-2, INF-γ), thus inhibiting embryo implantation. Th2 cells, on the contrary, produce anti-inflammatory cytokines (IL-4 and IL-10), which protect the embryo from the immune system assaults via suppression of Th1 cells.[67] Multiple studies found that the equilibrium between Th1/Th2 cytokines profile supported the feto-maternal immune tolerance during pregnancy.[68] Therefore, Th1 cells are predominantly produce negative effects on pregnancy, while, Th2 cell cytokines play an important part in induction and maintenance of pregnancy.[69]

Based on the numerous reports, the stem cell therapy is an efficient therapeutic modality in the treatment of unexplained infertility. Many clinical trials have evaluated the efficiency of stem cell therapies in humans. Use of the xenogeneic precursor stem cells as therapeutic agents has a number of advantages, such as relatively easy preparation, abundant sources, and preventable ethical issues.[62] After the implantation procedure, PSC are capable to survive, proliferate and differentiate into the finally differentiated cell, except placenta, ranging from hepatocyte, neural cells, muscles, liver, skin, and endocrine cells to oocytes and even sperm. Cultured in vitro, after implantation PSC proliferate in the recipient's body hence promoting tissue remodelling.[62]

In the meantime, administering organ-specific xenogeneic fetal precursor stem cells derived from the placenta and organs of the hypothalamus-pituitary-adrenals (HPA) and hypothalamus-pituitary-gonads (HPG) axis would help to restore and support normal production of the hormones regulating menstrual cycle, which facilitate timely ovulation and oocyte maturation for further successful conception and embryo development.[64,62] Stem cell transplantation as well as clinical use of decellularized organ-specific cell therapy products and cell derivatives becomes one of the most promising therapeutic solutions for incurable and untreatable diseases.[62]

Some studies reported benefits from the endometrial precursor stem cells, which are the inherent endometrial stem cells.[70] The engraftment of endometrial precursor stem cells has a great potential in the treatment of endometriosis. Thus, Tersoglio et al. found that the endometrial precursor stem cells implantation into the thinned endometrium allowed to achieve the higher rates of *In Vitro Fertilization* (IVF) and higher rates of successful pregnancy, especially in cases of repeated implantation failure and/or downregulation of estrogen receptors.[71]

More recently, the female Germ Precursor Stem cells and Ovarian Precursor Stem cells were discovered to be able to promote the ovarian regeneration and modulate the ovarian function. For instance, female germline precursor stem cells increased the amount of functional oocytes.[72]

Cellular extracts, procured from the cultured Precursor Stem Cells (PSC) are a well-established safe alternative. Clinical applications of cell extracts started in the beginning, mid- XX Century in Switzerland and Germany.[64,73,74] Stem cells are secreting a broad spectrum of paracrine factors, representing the components of the extracellular matrix, adhesion- and binding proteins, enzymes, growth factors, cytokines, and chemokines.[62,73,74]

Promising potential has been shown by organ-specific cell extracts, the cell-free therapeutic, which can be derived and procured from xenogeneic tissue, possess a great reliability and reproducibility, easily manufactured, packaged and transported, can be lyophilized or shock-frozen, and do not need to match the donor-recipient compatibility to avoid immune reactions.[73,74] Moreover, compared to stem cells, cell extracts have advantages of a lower production time and cost, a higher shelf-life, and relatively easy storage method.[73,74] Organ-specific cell extracts can be harvested from various types of cells and different culture conditions, to ensure the organ-specificity maintained. The evaluation of biologically active ingredients from various tissue sources has been done, showing the differences in the composition, peptide characterization and clinical effects produced.[75]

Different types of PSC secret a great variety of cytokines, chemokines, and growth factors that produce powerful paracrine effects on patient's endogenous pool of stem cells, as well as rejuvenating and regenerative effects on the tissue and organs. They also stimulate cell migration, proliferation, and tissue revascularization, thus promoting the organ's regeneration. One of the studies has demonstrated that mRNA expressions of interleukins IL-1β, IL-6, and IL-8 were significantly downregulated compared to the controls.[76] Few studies have demonstrated the more satisfactory outcomes in terms of endometrial growth and gestation in patients with thin endometrium following the use of decellularized cell-based therapy protocols.[77,78]

Mitochondrial replacement therapy in infertility

Mitochondrial substitution therapies target enhancement and/or replacement of the mitochondria inside the oocytes of the patient.[79] Currently there are two different techniques developed: either a oocyte cytoplasmic transfer from donor oocytes to patients' oocytes[80] or a transfer of oocyte chromosomes attached to the meiotic spindle from the donor's oocytes to the recipient's oocytes.[81] Both of these mitochondria transfer technologies have comparable clinical outcomes, and results in healthy mitochondria identified in the offspring population, though with a slightly less ratio of healthy mitochondria.[82,83]

In cases when there are no mitochondrial dysfunction diagnosed in females with idiopathic infertility, the clinical success of mitochondrial transfer is not just realized due to mitochondrial replacement *per se*, but rather due to numerous other biologically-active factors present in the maternal mtRNA. The success of these treatments combined with absence of adverse reactions and safety for the embryo, should encourage the use of these techniques in the clinical practice of infertility treatment. A more recent clinical study confirmed that mitochondria replacement and substitution therapy using mitochondria obtained from the patient's own stem cells instead of the allogeneic oocytes is another promising treatment modality to improve embryo quality in patients with unexplained infertility.[84] According to the study, 52 women (age from 27 to 49 years) were treated, that resulted in 61.5% fertilization rate and 23.8% pregnancy success rate: 11 live births, 1 intrauterine fetal death, and 4 miscarriages. The average implantation rate and birth rate were 18.6% and 17.5%, correspondingly. The physical and cognitive development of all the babies born was normal, and no mtDNA mutations were detected.[84]

Apart from the mitochondrial transfer, there are other additional natural treatments, which combine the activities of a direct anti-

oxidant agents, such as melatonin, glutathione, SOD2, catalase that protect cells against oxidative stress.[85] Other antioxidants, such as vitamins C and E, as well as coenzyme Q10, provide additional advantages, and can be utilized before more complicated and invasive treatment modalities used.

Conclusion

Unexplained infertility is associated with various pathological conditions with no clear pathogenesis identified, hence with no straightforward guidelines regarding the suitable treatment options. However, the research on aging of the reproductive system provides emerging evidences that novel anti-aging and regenerative medicine and translational medicine modalities can give solutions to the problems related to infertility. Among such novel methods to preserve and restore fertility in women with unexplained infertility are various forms of cell-therapy. Cell therapy is an expanding field of Bio-regenerative medicine attempting to alleviate numerous diseases involving chronic systemic low-grade inflammation, insulin resistance, fibrosis, and diminishing pool of endogenous stem cells. Further characterization of the existing cell products, research of their mechanisms of action, and large-scale clinical trials are necessary to establishing cell therapy as an effective therapeutic option for the treatment of unexplained infertility.

The decreased capacity of defence mechanisms against oxidative stress, which is one of the hallmarks of aging, results in a loss of normal functions of the female reproductive system. Hence, the assisted reproductive techniques should be developed in a way to address infertility caused by both reproductive aging in general and oxidative stress in particular. The application of stem cells, precursor stem cells, cellular extracts, organ-specific peptides, exosomes et cetera in regenerative medicine has been well-known and accepted for their strong anti-inflammatory, immunomodulatory, antioxidant capacities and stimulatory reparative effects. In addition to conventional cell therapy, the therapeutic use of targeted organ-specific cell therapy modalities has been researched and developed as an effective method of addressing to a particular disorder depending on the conditions and individual status of the patient. In conclusion, further development of cell therapy protocols as a promising treatment modality against unexplained infertility with or without the assisted reproduction technologies, and further research and development of therapeutic methods of attenuating the mitochondrial dysfunction in aging female reproductive system is required.

Acknowledgments

None.

Author contributions

Conceptualization: D.K., M.Y.; methodology: D.K.; writing—original draft preparation: M.Y., D.K.; writing—review and editing: D.K., M.Y.; supervision: D.K., M.W.; project administration: D.K., M.W.; funding acquisition: D.K., M.C. All authors have read and agreed to the published version of the manuscript.

Funding

None.

Conflicts of interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Stentz NC, Koelper N, Barnhart KT, et al., Infertility and mortality, *Am J Obstet Gynecol*. 2020;222(3):251.e1–e10.
2. Medicine, PCotASfR. Definitions of infertility and recurrent pregnancy loss: a committee opinion. *Fertil Steril*. 2013;99(1):63.
3. Tarek A Gelbaya, Potdar N, Jeve YB, et al. Definition and epidemiology of unexplained infertility. *Obstet Gynecol Surv*. 2014;69(2):109–115.
4. Cadenas E, Davies KJA. Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med*. 2000;29(3–4):222–230.
5. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev*. 2014.
6. Norbury CJ, Zhivotovsky B. DNA damage-induced apoptosis. *Oncogene*. 2004;23:2797–2808.
7. Zhang Y, Marcillat O, Giulivi C, et al. The oxidative inactivation of mitochondrial electron transport chain components and ATPase. *J Biol Chem*. 1990;265(27):16330–16336.
8. Passos JF, Saretzki G, Von Zglinicki T. DNA damage in telomeres and mitochondria during cellular senescence: Is there a connection? *Nucleic Acids Res*. 2007;35(22):7505–7513.
9. Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. *Cell*. 2005;120(4):483–495.
10. Tiosano D, Mears JA, Buchner DA. Mitochondrial dysfunction in primary ovarian insufficiency. *Endocrinology*. 2019;160(10):2353–2366.
11. Lampiao F. Free radicals generation in an in vitro fertilization setting and how to minimize them. *World J Obstet Gynecol*. 2012;1:29.
12. Lean SC, Derricott H, Jones RL, et al. Advanced maternal age and adverse pregnancy outcomes: A systematic review and meta-analysis. *PLoS ONE*. 2017;12(10):e0186287.
13. American Society for Reproductive Medicine. Assisted Reproductive Technologies (booklet). In Patient Fact Sheets and Booklets on Reproductivefacts.org; American Society for Reproductive Medicine: Birmingham, AL, USA, 2018.
14. Seifer DB, DeJesus V, Hubbard K. Mitochondrial deletions in luteinized granulosa cells as a function of age in women undergoing in vitro fertilization. *Fertil Steril*. 2002;78:1046–1048.
15. Tatone C, Carbone MC, Falone S, et al. Age-dependent changes in the expression of superoxide dismutases and catalase are associated with ultrastructural modifications in human granulosa cells. *Mol Hum Reprod*. 2006;12:655–660.
16. Perez GI, Jurisicova A, Matikainen T, et al. A central role for ceramide in the age-related acceleration of apoptosis in the female germline. *FASEB J*. 2005;19(7):860–862.
17. Gordo AC, Rodrigues P, Kurokawa M, et al. Intracellular calcium oscillations signal apoptosis rather than activation in in vitro aged mouse eggs. *Biol Reprod*. 2002;66;1828–1837.
18. Practice Committee of the American Society for Reproductive. Aging and infertility in women. Fertil. Steril. 2006, 86, S248–S252.
19. Cimadomo D, Fabozzi G, Vaiarelli A, et al. Impact of maternal age on oocyte and embryo competence. *Front Endocrinol*. 2018;9:327.
20. Prasad S, Tiwari M, Pandey AN, et al. Impact of stress on oocyte quality and reproductive outcome. *J Biomed Sci*. 2016;23:36.
21. Barritt JA, Brenner CA, Cohen J, et al. Mitochondrial DNA rearrangements in human oocytes and embryos. *Mol Hum Reprod*. 1999;5(10):927–933.

22. Bartmann AK, Romão GS, Ramos EdS, et al. Why do older women have poor implantation rates? A possible role of the mitochondria. *J Assist Reprod Genet*. 2004;21:79–83.
23. Wilding M, De Placido G, De Matteo L, et al. Chaotic mosaicism in human preimplantation embryos is correlated with low mitochondrial membrane potential. *Fertil Steril*. 2003;79(2):340–346.
24. Murakoshi Y, Sueoka K, Takahashi K, et al. Embryo developmental capability and pregnancy outcome are related to the mitochondrial DNA copy number and ooplasmic volume. *J Assist Reprod Genet.* 2013;30(10):1367–1375.
25. Chappel S. The role of mitochondria from mature oocyte to viable blastocyst. *Obstet Gynecol Int*. 2013.
26. Babayev E, Wang T, Szigeti-Buck K, et al. Reproductive aging is associated with changes in oocyte mitochondrial dynamics, function, and mtDNA quantity. *Maturitas*. 2016:93:121–130.
27. Chan CC, Liu VW, Lau EY, et al. Mitochondrial DNA content and 4977 bp deletion in unfertilized oocytes. *Mol Hum Reprod*. 2005;11(12):843–846.
28. Steuerwald N, Barritt JA, Adler R, et al. Quantification of mtDNA in single oocytes, polar bodies and subcellular components by real-time rapid cycle fluorescence monitored PCR. *Zygote*. 2000;8(3):209–215.
29. Fragouli E, Spath K, Alfarawati S, et al. Altered levels of mitochondrial DNA are associated with female age, aneuploidy, and provide an independent measure of embryonic implantation potential. *PLoS Genet*. 2015;11:e1005241.
30. Kinugawa C, Murakami T, Okamura K, et al. Telomerase activity in normal ovaries and premature ovarian failure. *Tohoku J Exp Med*. 2000;190(3):231–238.
31. Lim J, Luderer U. Oxidative damage increases and antioxidant gene expression decreases with aging in the mouse ovary. *Biol Reprod*. 2011;84(4):775–782.
32. Sasaki H, Hamatani T, Kamijo S, et al. Impact of oxidative stress on age-associated decline in oocyte developmental competence. *Front Endocrinol*. 2019;10:811.
33. Behrman HR, Kodaman PH, Preston SL, et al. Oxidative stress and the ovary. *J Soc Gynecol Investig*. 2001;8(1 Suppl Proceedings):S40–S42.
34. Fujii J, Iuchi Y, Okada F. Fundamental roles of reactive oxygen species and protective mechanisms in the female reproductive system. *Reprod. Biol Endocrinol*. 2005;3:43.
35. Kumar M, Pathak D, Venkatesh S, et al. Chromosomal abnormalities & oxidative stress in women with premature ovarian failure (POF). *Indian J Med Res*. 2012;135(1):92–97.
36. Hilali N, Vural M, Camuzcuoglu H, et al. Increased prolidase activity and oxidative stress in PCOS. *Clin Endocrinol*. 2013;79(1):105–110.
37. Al-Gubory KH, Garrel C, Faure P, et al. Roles of antioxidant enzymes in corpus luteum rescue from reactive oxygen species-induced oxidative stress. *Reprod Biomed Online*. 2012;25(6):551–560.
38. Ghafourifar P, Richter C. Nitric oxide synthase activity in mitochondria. *FEBS Lett*. 1997;418(3):291–296.
39. Lu J, Wang Z, Cao J, et al. A novel and compact review on the role of oxidative stress in female reproduction. *Reprod Biol Endocrinol*. 2018;16:80.
40. Rizk B, Badr M, Talerico C. Oxidative stress and the endometrium. *Studies on Women's Health*. In: Agarwal A, Aziz N, Rizk B, editors. Totowa, NJ, USA: Humana Press; 2013:61–74.
41. Burton GJ, Yung HW, Cindrova-Davies T, et al. Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and early onset preeclampsia. *Placenta*. 2009;30 (Suppl. A):S43–S48.
42. Trimarchi JR, Liu L, Porterfield DM, et al. Oxidative phosphorylation-dependent and -independent oxygen consumption by individual preimplantation mouse embryos. *Biol Reprod*. 2000;62:1866–74.
43. Chronopoulou E, Harper JC. IVF culture media: Past, present and future. *Hum Reprod. Update*. 2015;21:39–55.
44. Ortega I, Duleba AJ. Resveratrol is a natural polyphenol synthetized by several plants. *Ann NY Acad Sci*. 2015;1348:86–96.
45. Neves AR, Lucio M, Lima JLC, et al. Resveratrol in medicinal chemistry: a critical review of its pharmacokinetics, drug-delivery, and membrane interactions. *Curr Med Chem*. 2012;19:1663–1681.
46. Aquino CI, Nori SL. Complementary therapy in polycystic ovary syndrome. *Transl Med @ UniSa*. 2014;9:56–65.
47. Kolahdouz Mohammadi R, Arablou T. Resveratrol and endometriosis: In vitro and animal studies and underlying mechanisms (Review). *Biomed Pharmacother*. 2017;91:220–228.
48. Borra MT, Smith BC, Denu JM. Mechanism of human SIRT1 activation by resveratrol. *J Biol Chem*. 2005;280(17):17187–17195.
49. Hussain AR, Uddin S, Bu R, et al. Resveratrol suppresses constitutive activation of AKT via generation of ROS and induces apoptosis in diffuse large B cell lymphoma cell lines. *PLoS ONE*. 2011;6(9):e24703.
50. Manna SK, Mukhopadhyay A, Aggarwal BB. Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-_B, Activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. *J Immunol*. 2000;164:6509–6519.
51. Li N, Liu L. Mechanism of resveratrol in improving ovarian function in a rat model of premature ovarian insufficiency. *J Obstet Gynaecol Res*. 2018;44:1431–1438.
52. Wu M, Ma L, Xue L, et al. Resveratrol alleviates chemotherapy-induced oogonial stem cell apoptosis and ovarian aging in mice. *Aging (Albany NY)*. 2019;11(3):1030–1044.
53. Ortega I, Duleba AJ. Ovarian actions of resveratrol. *Ann NY Acad Sci*. 2015;1348:86–96.
54. Raizner AE. Coenzyme Q10. *Methodist Debakey Cardiovasc J*. 2019;15:185–191.
55. James, AM, Smith RAJ, Murphy MP. Antioxidant and prooxidant properties of mitochondrial Coenzyme Q. *Arch Biochem Biophys*. 2004;423(1):47–56.
56. Özcan P, Fıçıcıoglu C, Kizilkale O, et al. Can Coenzyme Q10 supplementation protect the ovarian reserve against oxidative damage? *J Assist Reprod Genet*. 2016;33:1223–1230.
57. Akarsu S, Gode F, Isik AZ, et al. The association between coenzyme Q10 concentrations in follicular fluid with embryo morphokinetics and pregnancy rate in assisted reproductive techniques. *J Assist Reprod Genet*. 2017;34(5):599–605.
58. Xu Y, Nisenblat V, Lu C, et al. Pretreatment with coenzyme Q10 improves ovarian response and embryo quality in low-prognosis young women with decreased ovarian reserve: A randomized controlled trial. *Reprod Biol Endocrinol*. 2018;16(1):29.
59. Florou P, Anagnostis P, Theocharis P, et al. Does coenzyme Q10 supplementation improve fertility outcomes in women undergoing assisted reproductive technology procedures? A systematic review and meta-analysis of randomized-controlled trials. *J Assist Reprod Genet*. 2020;37(10):2377–2387.
60. Chan YM, Bailey R, O'Connor DL. Folate. *Adv Nutr*. 2013;4(1):123–125.
61. Kurpad AV, Anand P, Dwarkanath P, et al. Whole body methionine kinetics, transmethylation, transulfuration and remethylation during pregnancy. *Clin Nutr*. 2014;33(1):122–129.

62. Klokol D, Chan MKS. Stem Cells in regenerative medicine: Carpe diem, carpe vitum. Troubador, UK. 2019. 495 p.

63. Klokol D, Chan MKS, Wong MBF. European wellness – the evidenced rationale behind the biological medicine: ad astra per aspera. *J Pharm Biomed Sci*. 2017;07(2):19–22.

64. Klokol D, Nallenthiran L, Wong MB, et al. Biohormonal revitalization therapy from the perspective of biological regenerative medicine: The evaluation of premature menopause and andropause treatment outcomes in longitudinal cohort study. *Obstetrics & Gynecology International Journal*. 2019;10(4):236–241.

65. 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.

66. Hashii K, Fujiwara H, Yoshioka S, et al. Peripheral blood mononuclear cells stimulate progesterone production by luteal cells derived from pregnant and non-pregnant women: possible involvement of interleukin-4 and interleukin-10 in corpus luteum function and differentiation. *Hum Reprod*. 1998;13(10):2738–2744.

67. Mjösberg J, Berg G, Jenmalm MC, et al. FOXP3+ regulatory T cells and T helper 1, T helper 2, and T helper 17 cells in human early pregnancy decidua. *Biol Reprod*. 2010;82(4):698–705.

68. Chaouat G. Innately moving away from the Th1/Th2 paradigm in pregnancy. *Clin Exp Immunol*. 2003;131(3):393–395.

69. Li XC, Zand MS, Li Y, et al. On histocompatibility barriers, Th1 to Th2 immune deviation, and the nature of the allograft responses. *J Immunol*. 1998;161(5):2241–2247.

70. Tempest N, Maclean A, Hapangama DK. Endometrial stem cell markers: current concepts and unresolved questions. *Int J Mol Sci*. 2018;19(10):3240.

71. Tersoglio AE, Tersoglio S, Salatino DR, et al., Regenerative therapy by endometrial mesenchymal stem cells in thin endometrium with repeated implantation failure. A novel strategy. *JBRA Assisted Reproduction*. 2020;24(2):118–127.

72. Grieve KM, McLaughlin M, Dunlop CE, et al. The controversial existence and functional potential of oogonial stem cells. *Maturitas*. 2015;82(3):278–281.

73. Klokol D, Lingeswran Nallenthiran, Mike KS Chan, et al. Cell therapy as the main stratagem of anti-aging and regenerative medicine. *Europ Journ Pharm Med Res*. 2019;6:295–299.

74. Klokol D, Nallenthiran L, Michelle BF Wong, et al. Live cell therapy: historical aspects, mechanisms of action, safety and success stories. *J Stem Cell Res Ther*. 2019;5:38–42.

75. Good A, Wells A, Katz B, et al. MALDI-ToF Analysis of Mitochondrial Peptides. *Clinical Medicine Insights*. 2022;3(2):297–303.

76. Metcalf E. The effect of platelet-rich plasma (PRP) on intraluminal fluid and pregnancy rates in mares susceptible to persistent mating-induced endometritis (PMIE). *Journal of Equine Veterinary Science.* 2014;34(1):128.

77. 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(1):1286–1290.

78. Tandulwadkar SR, Naralkar MV, Surana AD, et al. Autologous intrauterine platelet-rich plasma instillation for suboptimal endometrium in frozen embryo transfer cycles: a pilot study. *J Hum Reprod Sci*. 2017;10(3):208–212.

79. Morimoto Y, Gamage USK, Yamochi T, et al. Mitochondrial transfer into human oocytes improved embryo quality and clinical outcomes in recurrent pregnancy failure cases. *Int J Mol Sci*. 2023;24(3):2738.

80. Cohen J, Scott R, Schimmel T, et al. Birth of infant after transfer of anucleate donor oocyte cytoplasm into recipient eggs. *Lancet*. 1997;350(9072):186–187.

81. Tesarik J, Nagy ZP, Sousa M, et al. Fertilizable oocytes reconstructed from patient's somatic cell nuclei and donor ooplasts. *Reprod Biomed Online*. 2001;2:160–164.

82. Barritt JA, Brenner CA, Malter HE, et al. Mitochondria in human offspring derived from ooplasmic transplantation. *Hum Reprod*. 2001;16:513–516.

83. Tesarik J. Purifying selection on mitochondrial DNA in maturing oocytes: Implication for mitochondrial replacement therapy. *Hum Reprod*. 2017;32(9):1948–1950.

84. Morimoto Y, Gamage USK, Yamochi T, et al. Mitochondrial transfer into human oocytes improved embryo quality and clinical outcomes in recurrent pregnancy failure cases. *Int J Mol Sci*. 2023;24:2738.

85. Tesarik J, Galán-Lázaro M, Mendoza-Tesarik R. Ovarian aging: molecular mechanisms and medical management. *Int J Mol Sci*. 2021;22(3):1371.

Citation: Yemeliyanova M, Chan MKS, Wong MBF, et al. Unexplained infertility: a fresh look at the old problem and the novel therapeutic options of its treatment. *Obstet Gynecol Int J.* 2024;15(1):6–12. DOI: 10.15406/ogij.2024.15.00726