Chapter 10

Cryopreservation of Human Gametes and Embryos: Current State and Future Perspectives

Jeseta Michal, Zakova Jana, Ventruba Pavel,
Bartosz Kempisty and Crha Igor

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64950

Abstract

Cryopreservation of human gametes and embryos is an important and widely used method in most embryology laboratories. During last years, the practice of single embryo transfer was a greater demand for reliable cryostorage of surplus embryos. Currently, there are two basic principally different methods usable for cryopreservation: slow freezing and vitrification. Vitrification is a very promising method with massive use in embryology. Nowadays, this method is also suitable for cryopreservation of human mature oocytes (one of the most problematic cell in cryobiology). This progress in the field of cryopreservation opens new perspectives in assisted reproduction. Recent effective oocyte vitrification systems have a significant impact on clinical practice. This chapter gives a view of human gametes (sperms, oocytes) and embryos cryopreservation application and possibilities. Indications and methods of cryopreservation and thawing are mentioned.

Keywords: gamete, oocyte, spermatozoa, embryo, cryopreservation, assisted reproduction, vitrification

1. Introduction

Cryopreservation of human gametes and embryos is very important method in most embryology laboratories. Two basic cryopreservation techniques rule the field, slow-rate freezing (first developed) and vitrification, which have gained a foothold in recent years. Vitrification is relatively simple, requires no expensive programmable freezing equipment and uses a small

amount of liquid nitrogen for freezing. Vitrification of human oocytes and embryos (especially at early stages) is more effective than slow freezing.

During last years, the practice of single embryo transfer was a greater demand for reliable cryostorage of surplus embryos. The first reports of successful freezing and thawing of human embryos were in 1983 [1]. There are a growing number of indications for oocyte cryopreservation as oocyte donation, fertility preservation for cancer patients or social egg freezing. Reproductive behaviour of women has been changed in last years. There is a delay in the age of motherhood due to various reasons like career, live style or education. It is known, that in women older than 35 years, reduction of ovarian reserve is observed. The use of younger cryopreserved oocytes can reduce the risk of foetal loss and aneuploidies associated with ageing oocytes. Oocyte cryopreservation simplifies the logistics of assisted reproductive technology (ART) cycles in donation programme, and there is no need for menstrual cycle synchronization between donor and recipient.

Damage of reproductive function is very frequent and well documented side effect associated with the treatment of malignant tumours. The increasing success of cancer treatment and determined efforts to improve the quality of life after successful treatment has turned attention to the preservation of reproductive function in young women and also in young men. Sperm freezing is largely recommended to preserve fertility prior to the oncology treatment. Cryopreservation of spermatozoa is routinely used in a variety of reasons (sperm bank, donor programme, etc.).

For this reasons, cryopreservation of gametes and embryos is more and more important part of human-assisted reproduction.

2. Cryopreservation: principles and methods

2.1. Cellular cryotolerance

Cryopreservation of cells and their storage in liquid nitrogen at −196°C is not physiological process. The freezing process can cause stress and mechanical damage of cells by ice crystal formation [2]. Cell damage can occur at any time during cryopreservation process. Cell lysis can be induced by intracellular ice formation. This major change is easily observed through routine microscopic observations. However, damages can also occur in the cellular structural/functional levels involving intracellular organelle changes, what is more difficult to diagnose.

Three types of damage during cooling process in oocytes and embryos were described:

- the damage of microtubules, including meiotic spindle with relative high temperature (from +5 to −5°C);
- intracellular crystal formation (temperature from −5 to −80°C);
- damage of zona pellucida or the cytoplasm (temperature from −80 to −150°C).

http://dx.doi.org/10.5772/64950

The using of cryoprotectants makes a damage of oocytes via cytotoxic and osmotic effect. The addition and the removal of cryoprotectants from the oocyte create an osmotic imbalance across the oocyte membrane, which may result in large volumetric changes and cause damages in the cell morphology, cytoskeletal structures and physiologic function.

Protein structure and function, as well as metabolism, can also be affected. Cells require a period of recovery after thawing, and then, they are able to continue normal intracellular function.

Cryopreservation affects various organelles like intracellular lipids [3], mitochondria (**Figure 1**) cortical granules [4], cytoskeletal structure, zona pellucida and also meiotic spindle [5]. Deleterious effects of meiotic spindle can resulted into chromosome disaggregation. Improper chromosome segregation could lead to aneuploidy and genetic errors, which may cause embryonic and foetal abnormalities. Furthermore, cryopreservation can induce releasing of cortical granule what makes changes of zona pellucida (zona hardening) [4].

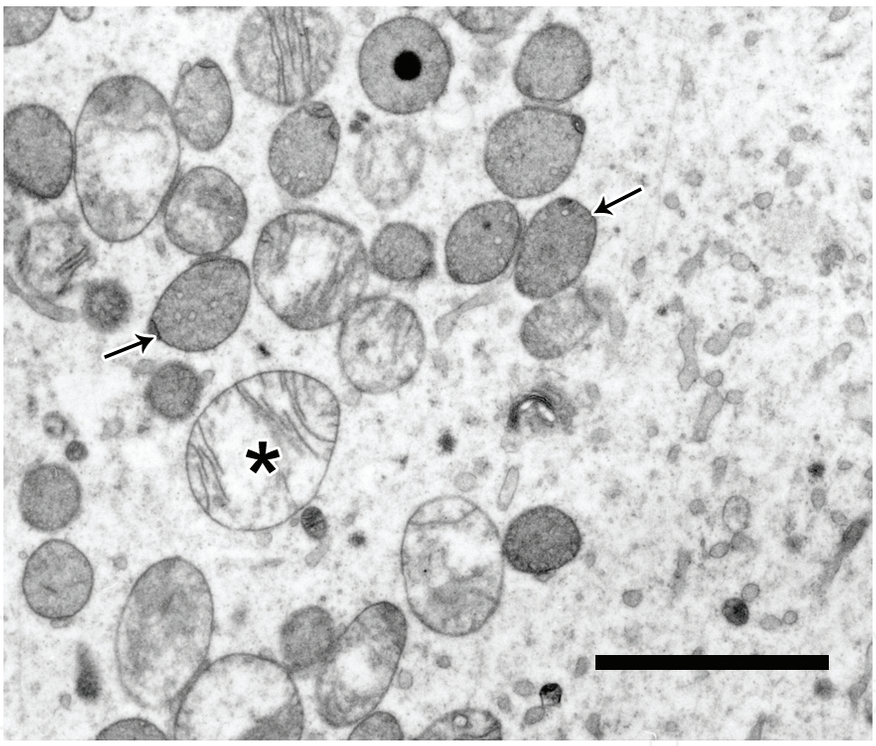

Figure 1. Mitochondria from primary oocyte after cryopreservation with morphological alterations: swollen mitochondria (*) and mitochondria with atypical tubular cristae (black arrow). Scale bar represents 2 μm.

2.2. Cryoprotectants

Cryoprotectants are substances with high solubility and cytotoxicity, what is directly proportional to their concentration and temperature. They aim to protect cells from any damage what is known as cold shock, during freezing-warming procedure. Cryoprotectants bond water and they reduce the toxic effect of high concentrations of other compounds. At high concentrations,

cryoprotectants minimize the damage caused by ice formation, as they cause the water to form a glass rather than ice crystal. After thawing, the cryoprotectants must be removed from cells to avoid their deleterious effect on further fertilization and embryonic development.

2.2.1. Membrane permeable cryoprotectants

These solutions displace water via an osmotic gradient and partly occupy the place of the intracellular water. Indeed, increase in the extracellular osmolarity generates an osmotic gradient across the cell membrane-supporting dehydration of the cell. In this group, compounds are with relatively low molecular weight (<100 g/mol). The most commonly used cryoprotectants for oocyte and embryos are ethylene glycol, 1,2-propandiol and dimethyl sulfoxide (DMSO) [6]. Ethylene glycol is widely used during the vitrification of human oocytes and embryos due to its low toxicity and high permeability. In present time, it is standard part of all vitrification protocols. During equilibration step especially in oocyte vitrification, the compounds of very high concentration (>4 M concentration) are used.

2.2.2. Membrane nonpermeable cryoprotectants

Nonpermeable cryoprotectants are usually large molecules, which remain in extracellular solution. Extracellular saccharides and macromolecules (sucrose, trehalose, Ficoll, PVP) are commonly added to vitrification solutions. They help draw water out of the blastocoel to attain better dehydration and reduce osmotic shock. Very frequent approach is combination of more cryoprotectants for decreasing the individual specific toxicity of each solution. At least, one of these cryoprotectants should be permeable (with higher toxicity) and one or two nonpermeable (lower toxicity) [7].

For example, during vitrification commonly used ethylene glycol or DMSO or propanediol (permeable) are often combined with sucrose or PVP (nonpermeable), which reduce the concentration of permeable cryoprotectants and facilitate dehydration and vitrification.

Cell permeability is an important factor for determining the conditions for cryopreservation. The permeability of mouse embryos increases as development proceeds to the compacted morula. Ethylene glycol is less permeating than propylene glycol at the one cell stage. In morula stage, ethylene glycol is far more permeating than other cryoprotectants. Exchange of water and cryoprotectants in expanded pig blastocyst occurs predominantly by facilitated diffusion but in oocytes predominantly by simple diffusion [8]. This was related to the expression of aquaporin three mRNA, which was abundantly active in expanded blastocyst, but not in oocytes. The common consensus is that rapidly permeating agents are favoured for oocyte cryopreservation, because the exposure time before cooling can be shortened, and because osmotic swelling during removal of the cryoprotectant can be minimized.

2.3. Slow freezing

This technique involves stepwise programmed decrease in temperature. The procedure is lengthy and requires the using of expensive equipment (**Figure 2**). This process does not exclude ice crystal formation, which can have extremely deleterious effects [9].

http://dx.doi.org/10.5772/64950

Figure 2. Programmable cryo freezer Planer Kryo F10.

Slow freezing is a technique with long history but in comparison to vitrification actually does not bring any advantages. Vitrification methods are more efficient and reliable than any version of slow freezing [10]. After a long period of practising was the convention slow freezing method completely stopped in many centres and was replaced by routine vitrification.

2.4. Vitrification

Limiting factor for all cryopreservation methods is ice crystal formation that drastically reduced survival of embryos and oocytes. Vitrification process produces a glasslike solidification of living cells, which completely avoids ice crystal formation. It is well known that vitrification requires a greater amount of cryoprotectants, what increases the toxicity of their environment. However, it was claimed higher survival rate after using vitrification instead of slow freezing [11]. Vitrification is very simple, cost-effective process, but the skills to perform require good manual training.

3. Gamete cryopreservation

3.1. Spermatozoa cryopreservation

Cryopreservation of human semen is well-established laboratory procedure to maintain the fertilizing potential of spermatozoa during storage in liquid nitrogen. Modern trends in assisted reproduction technologies influenced the indications for human sperm cryopreservation. Spermatozoa are not so sensitive to cryopreservation damage (in comparison with other

cell), because of the high fluidity of the membrane and the low water content (about 50%). The effect of cryopreservation on sperm DNA integrity is still unclear. There is no agreement in literature on whether or not affect cryopreservation sperm chromatin integrity.

When clinicians became aware that azoospermia or very severe oligozoospermia could not be improved by medical treatment, it arises the idea to create sperm banks. Today, cryopreservation of spermatozoa is routinely used in a variety of reasons:

1. Donor or husband semen storage for assisted reproduction.
2. Sperm banking for husband sperm for psychological or other reasons (it is not always possible to produce sperm samples at the appropriate time in the cycle).
3. Storage of epididymal or testicular spermatozoa after MESA/TESE, to avoid repeated biopsies or aspirations.
4. Storage of sperm as a fertility "insurance" for future.
5. Preservation of semen before surgical, chemical or radiological cancer therapy, which may lead to testicular failure or ejaculatory dysfunction. Also other nonmalignant diseases, such as diabetes or autoimmune disorders, may lead to testicular damage.
6. Male gamete freezing is largely recommended to preserve fertility in those subjects who are exposed to potentially toxic agents, which may interfere with gametogenesis.

Semen preservation before the beginning of therapy should be proposed to all adult men and postpubertal boys. To date, no clinically proven methods are available to preserve fertility in prepubertal males. The testicular cancer survivors have a good chance of fathering a child by using sperm cryopreserved prior to the oncology treatment thanks to assisted reproduction methods [12].

In the ICSI era, almost all cryopreserved semen sample, even when it contains only few sperm, could be used for subsequent infertility treatment. Genetic damage is unknown.

Cryopreservation is known to cause some changes in sperm morphology, including damage to mitochondria, the acrosome and the sperm tail. The sperm motility is particularly sensitive, and it is generally accepted that it can be reduced to 50% after the cryopreservation/thawing procedure. Due to this fact, it is necessary to choose potential donors with an emphasis on this sperm parameter.

3.2. Oocyte cryopreservation

Cryopreservation of human oocyte can be an alternative to circumvent many of the ethical issues associated with embryo cryopreservation. For oocyte cryopreservation, it is very suitable to use vitrification method. Oocyte cryobanking is a new more efficient approach in oocyte donor-recipient treatment. On the basis of guideline from the Practice Committees of the American Society for Assisted Reproductive Medicine (from 2013) and, in March 2012, European Society of Human Reproduction and Embryology (ESHRE), it is indicated that mature oocyte vitrification and warming are not experimental and should no longer be

http://dx.doi.org/10.5772/64950

considered as experimental procedures. This progress in the field of cryopreservation opens new perspectives in assisted reproduction. Recent effective oocyte vitrification systems have a significant impact on clinical practice. It is a possible way in countries where the law forbids the cryopreservation of embryos. Indeed, efficient oocyte vitrification technology eliminates synchronization between donor and recipient. It enables the establishment of egg banks by eliminating the logistics of coordinating egg donors with their recipients. Progress in oocyte vitrification brings new possibilities mainly for women, who are trying to postpone child-bearing from professional or social reasons. The process was originally developed as a way to preserve the fertility of cancer patients undergoing possibly sterilizing chemotherapy, and it is relatively simple.

Oocyte cryopreservation is less successful than embryo cryopreservation for many reasons. Oocytes have small surface to volume ratio, temperature-sensitive metaphase spindle [13], zona pellucida as very specific structure and susceptibility to parthenogenetic activation. Oocytes are one of the biggest cells with high likelihood of intracellular ice formation [14] Oocytes are very unique cells, because of their developmental capacity to be fertilized and then to support early embryonic development. This capacity derives from maternal legacy of the myriad of transcript, proteins and energetic substrates and also cytoplasmic organelles, which facilitate early mitotic divisions of the embryo until embryonic genome activation occurs [15]. This highly organized structure often incurs serious damage after cryopreservation. The volume of mammalian oocyte is much bigger than that of spermatozoa, thereby substantially decreasing the surface to volume ratio and making them sensitive to chilling and highly susceptible to intracellular ice formation. In fact, in a developing embryo, cleavage division occurs without any increase in volume until blastocyst stage, leading to higher nucleus-cytoplasmic ratio of embryo blastomeres compared with the oocyte. Oocytes are substantially more prone to cryo damage than are embryos. Number of blastomeres in early embryos provides great flexibility to compensate for any detrimental effects of cryopreservation, because missed blastomeres can be replaced by the daughter cells of dividing intact ones. Oocytes contain one-half of the genetic material of the future individual, and so any damage to its chromatin structure may result in deleterious defects in the developmental competence of the resulting embryos. Damage of meiotic spindle can result in chromosomal abnormalities after thawing. The permeability of oocyte plasma membrane to cryoprotective agents is low compared with embryo [6].

Although mature oocytes in metaphase II are sensitive to cryopreservation (detrimental effect on meiotic spindle or premature cortical granule release) and immature oocytes on prophase I (GV oocytes) look that are more suitable for cryopreservation. It is well known that oocytes frozen at GV stage exhibited decreased affectivity of *in vitro* maturation and increased spontaneous parthenogenetic activation [16]. For this reason in case of immature oocytes, it is recommended to use *in vitro* maturation and after that perform their subsequent vitrification.

It was presented that highly organized structure of fresh oocyte changes dramatically (at cellular, ultrastructural, molecular and developmental levels) after cryopreservation. Cryo-preserved oocytes have cellular characteristics that differ from those of the fresh oocytes.

3.2.1. Cryopreservation of ovarian tissue

Fertility preservation has a great importance to many young women with cancer [17]. Cryopreservation of ovarian tissue is a safe, simple and effective option for preserving fertility in young patients facing or undergoing gonadotoxic therapy. Oocytes in primordial follicles are very small and tolerate cryopreservation very well. The removal of ovarian tissue is a simple procedure. Ovarian tissue can be obtained using minimally invasive techniques during laparoscopy, with unilateral ovariectomy or partial ovariectomy. Ovarian tissue can be cryopreserved independently of the menstrual phase.

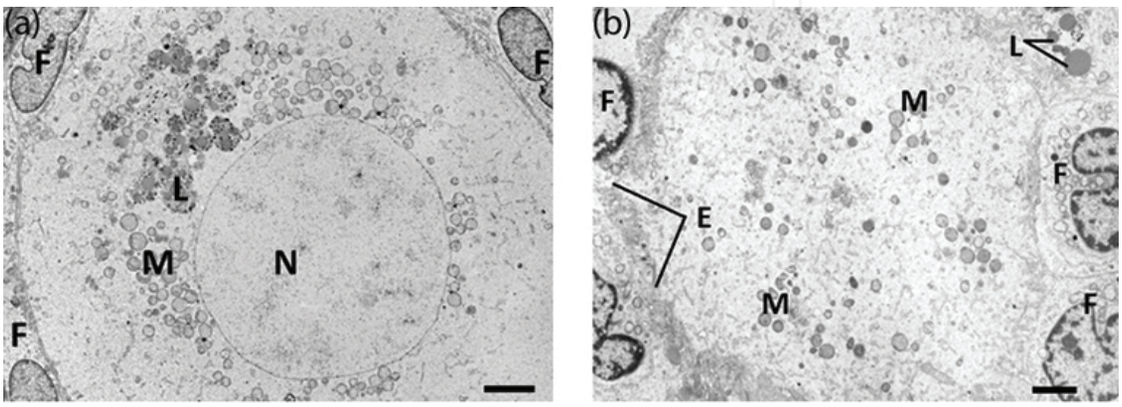

Figure 3. Primary follicle from ovarian cortex before (a) and after (b) cryopreservation with morphological alterations. The oolemma of oocyte after cryopreservation is more undulated and interrupted (E), and the cytoplasm of follicular cells (F) is vacuolated. N, nucleus; M, mitochondria; L, lipid droplets. Scale bar represents 5 μm [19].

In 2004, first live birth after autotransplantation of human ovarian tissue was reported [18]. To date, 60 live births have been reported worldwide following transplantation of cryopreserved ovarian tissue. However, research on the cryopreservation of ovarian tissue as a method of fertility preservation has now been continuing for more than a decade, and considerable successes have recently been achieved.

In centres that offer cryopreservation of ovarian tissue, the procedure can be performed one day after the patient's first visit. After the tissue has been removed, it can be processed immediately or transferred in special transportation containers to a centre specializing in the cryopreservation of ovarian tissue, with an associated cryobank (**Figure 3**).

4. Zygote and embryo cryopreservation

Cryopreservation of human embryos is a safe procedure, which has been carried out for more than last 30 years. In development of *in vitro* techniques and together with single embryo transfer becoming greater demand for an efficient and reliable cryopreservation method for surplus embryos. It is possible to cryopreserve the human zygotes immediately after fertilization, at the pronuclear stage or embryos during early cleavage stages (2–8 cells) or at the expanded blastocyst stage (after 5–7 days in culture).

http://dx.doi.org/10.5772/64950

Embryos are cryopreserved in any embryonic stages. Still there does not exist a common consensus what is the most optimal developmental stage for embryo cryopreservation.

Since morphology of vitrified and thawed embryos is not enough to assess the viability, the possibility of culturing for a few more days before transfer can ensure that embryo is for transfer. In contrary to oocytes, embryos are after cortical reaction, which gives the ooplasmic membrane more stability to cope with the low temperature and osmotic changes.

4.1. Cryopreservation of zygotes

For cryopreservation of human zygotes, it is suitable to use only vitrification method. Slow freezing method has more than threefold worse results than vitrification [20].

The recent reported data for successful pregnancies suggested that the vitrification of human zygotes and early-stage embryos is a perfect alternative to slow freezing techniques especially in countries where cryopreservation of later stage human embryos is prohibited either by law or due to religious reasons.

4.2. Cryopreservation of cleaved embryos

Vitrification of early-stage human embryo is acceptable and better alternative than slow rate freezing because of the higher survival rate and increased rates of pregnancy. Cryopreservation of cleaved embryos is not so effective as cryopreservation of blastocyst.

4.3. Cryopreservation of blastocyst

Blastocysts are the top embryos, what have successfully passed the critical step of genomic activation and have a high developmental potential. Their advantage is containing numerous small cells; thus, the loss of some cells during freezing and thawing is probably less harmful for future development of the embryo. Furthermore, during extended cultivation, embryos with worse viability are arrested in development and will not be cryopreserved.

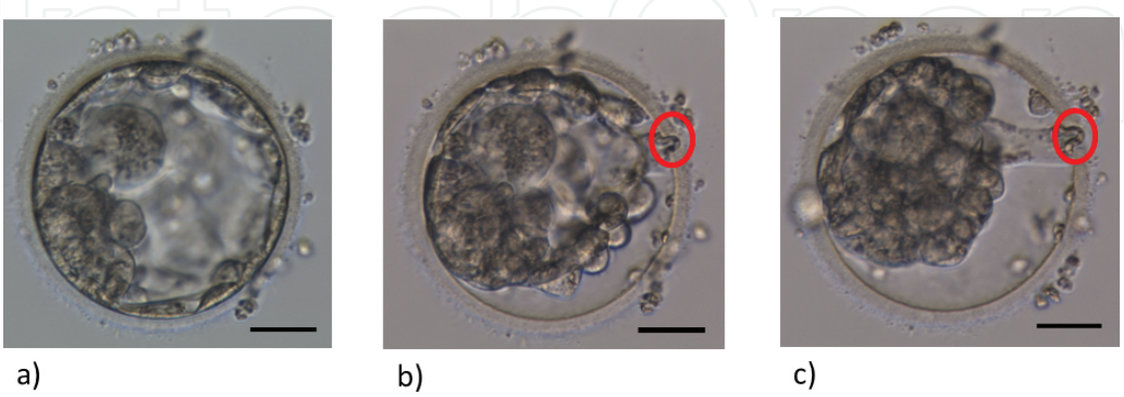

Figure 4. Laser blastocoel puncture (assisted shrinkage): human expanded blastocyst before puncture; (a) laser pulse open zona pellucida (red circle) and make a small defect in the trophectoderm (b), which resulted to blastocyst shrinking (c). Scale bars represent 30 μm.

Blastocyst presents special challenge to cryopreservation. Excessive water in the blastocoel may lead to ice formation and subsequent damaging of cellular structures. To minimize this risk, removal of some of the blastocoel fluid has been attempted. Removal of blastocoel fluids can be done by perforating the blastocoel and letting the fluid flow passively out [21]. The process called assisted shrinkage can be performed in a variety of ways, including microneedle puncture, repeated micropipetting of the blastocoel or laser-pulse opening of zona pellucida (**Figure 4**).

5. Our experiences with cryopreservation of gametes, embryos and tissues

We have cryopreserved sperm since 1991, and we introduced embryo freezing in 1995. In the beginning, we performed slow freezing by Planer, but from 2007, we prefer vitrification. Well-functioning cryopreservation was an essential prerequisite for a donation of gametes and embryos program. Within the centres of assisted reproduction, we had the first and the largest sperm bank in the Czech Republic from 1995 (currently, we have 100 donors).

We have also built a centre for fertility preservation for both male and female oncologic patients. Methods of preserving the fertility in young women can be divided into three cryopreservative methods: embryo-, mature oocyte- and ovarian tissue-cryopreservations. We have started as the first with ovarian tissue freezing and sperm freezing before gonadotoxic treatment in the Czech Republic. We cryopreserved ovarian tissue of 23 women before gonadotoxic treatment (from January 2006 to December 2015). During October 1995 to December 2015, we cryopreserved the sperm of 1231 men—oncologic patients (587—testicular cancer diagnosis). The testicular cancer survivors have a good chance of fathering a child by using sperm cryopreserved prior to the oncology treatment, even when it contains only limited number of spermatozoa. There are 41 patients in our centre, who returned for infertility treatment underwent 58 treatment cycles with cryopreserved sperm. Totally, 20 pregnancies were achieved, that is 34.5% pregnancy rate. The implementation of all young oncological patient sperm cryopreservation has an important place in our laboratory methods.

6. Trends and future perspectives

Many researchers are studying different methods to improve cryopreservation outcome by modification of essential factors (cryoprotectants, freezing rate, warming). Trends and new perspectivities in cryopreservation in human-assisted reproduction are an important part of this chapter.

6.1. Optimization of current methods

6.1.1. Inhibition of ROCK kinase

Several new steps and procedures for optimization of current methods were developed during last years. It is well known that vitrification procedure often increases apoptosis in embryonic

http://dx.doi.org/10.5772/64950

cells, and it results in decrease of developmental competence. Specific postvitrification treatment can suppress this effect in somatic cells or animal oocytes. It was reported that inhibition of Rho-associated coiled-coil kinase (ROCK) improves developmental competence of vitrified/thawed bovine oocytes [22]. This treatment was also effective in human embryonic cells [23], or bovine blastocysts [24]. ROCK kinase is involved in regulation of metabolism, apoptosis, growth, cytoskeletal assembly and cell contraction.

6.1.2. New vitrification devices

New way to increase the cooling rate is reducing the use of cryoprotectants consist in the reduction of liquid nitrogen temperature. In order to avoid vaporization of liquid nitrogen, the temperature is reduced until −210°C, applying a negative pressure [25]. In this condition, nitrogen partially solidifies and creating nitrogen slush, which is less likely to evaporate on contact with specimen compared to liquid nitrogen. This method was very effective in human blastocyst [26]. Cells immersed into nitrogen slush cool more rapidly because they come into contact with liquid nitrogen sooner than those immersed in normal liquid nitrogen. It can provide very high cooling rate (up to 135,000°C/min. The cooling rate is enhanced mainly in the first part of cooling (from 20 to −10°C).

6.1.3. Hydrostatic pressure

Survival of cryopreserved oocytes and embryos is affected by many factors, and their role is still unclear. Recent studies also reported promising results after applying of high hydrostatic pressure during pretreatment of oocytes and embryos. Some studies show that cultivation medium has a dramatic effect on efficiency of cryopreservation methods. However, it was tested that short time exposition of high hydrostatic pressure prior to vitrification (probably thought production of HSP proteins [27]) significantly improved the survival and hatching rate in murine blastocyst [28].

6.1.4. Antioxidative treatment

Oxidative stress has been implicated in many different types of cell injuries, including membrane peroxidation, oxidation of amino acids or nucleic acids, apoptosis and necrosis, which decrease survival rate after cryopreservation. Experiment realized in model animals indicated positive effect of the presence of antioxidant in cultivation medium after thawing of embryos [29]. Indeed, supplementation of α-tocopherol in recovery culture medium resulted in a significantly higher blastocyst yield from the postwarm bovine oocytes in comparison with control oocytes [30]. Actual methods are capable of achieving proper vitrification attaining high level of viscosity and dehydration and delivering high freezing and warming rates. Recent studies realized on experimental animals bring new applicable knowledge suitable for optimization of current method. In our opinion, further research in vitrification media and devices is important for next development of these methods.

It is well known that type of culture media (where are embryos after thawing) is very important for successful thawing process. This fact is often ignored and we believe that gentle appropriate treatment after thawing can improve the survivability of oocytes and embryos.

6.2. New trends

6.2.1. Freeze all

One of the new strategies is also "freeze all". In "freeze all cycle", all embryos are cryopreserved and later used after thawing in another reproductive cycle. This approach is very often used in cooperation with preimplantation genetic screening (PGS) of embryos before their transfer into the uterus. New trend in this approach is genetic screening of blastomere after biopsy at the fifth day of *in vitro* cultivation or later. There is no other way and all embryos must be cryopreserved and stored in liquid nitrogen. Embryos are thawed after final decision about embryo aneuploidy and their suitability for transfer into the uterus.

It was presented that implantation, clinical and ongoing pregnancy rates of ART cycles may be improved by performing cryoembryotransfer compared with fresh embryo transfer [31]. It can be explained by a better embryo endometrium synchrony achieved with endometrium preparation cycles. In frozen embryo transfers, endometrium priming may be achieved with the use of E_2 and P, and the endometrial development can be controlled more precisely than in cycles with gonadotropins [32].

6.2.2. Social freezing

Frozen oocyte replacement is a technique where oocytes are retrieved, frozen, stored and fertilized only after thawing them for transfer. This technique helps women to preserve the future ability of having genetically related children at later point in life. It was first used for cancer patients before chemotherapy or radiotherapy. However, it can be also used for delaying motherhood for any reason, such as an absence of suitable partner or a work career. Large companies like Facebook or Apple have recently included social freezing for female employees as an employment benefit. Indeed, just as for fresh oocyte, the outcome of IVF with vitrified oocytes is highly dependent on maternal age. The most appropriate age for effective cryopreservation is unknown, but ideally, it would be in the early to mid-30s, before age at which woman's fertility naturally declines. Younger women have higher chance that they will never require these eggs. Elder women can be under risk of insufficient procedure with few amounts of oocytes, aneuploidy oocytes and very low probability of pregnancy [33].

7. Conclusion

Finally, it is well known that the embryologist training would have a major bearing on the vitrification outcome. Further vitrification procedural improvements using postvitrification chemical treatment would reduce the high sensitivity of oocytes and embryos to cryopreservation and provide valuable information during an advanced postcryopreservation thawing

procedure. Furthermore, the strategic placement of embryonic culture media is very important for a successful freezing/thawing process. This fact is often ignored, and we have determined that gentle appropriate treatment after thawing can improve survivability of human oocytes and embryos in much the same manner as witch model animals.

Acknowledgements

Our work is supported by a grant MH CZ—DRO (FNBr, 65269705), and funds are received from the Faculty of Medicine, Masaryk University Brno, Czech Republic to junior researcher Michal Jeseta.

Author details

Jeseta Michal[1*], Zakova Jana[1], Ventruba Pavel[1], Bartosz Kempisty[2,3,4] and Crha Igor[1]

*Address all correspondence to: jeseta@gmail.com

1 Department of Obstetrics and Gynaecology, Center of Assisted Reproduction, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic

2 Department of Histology and Embryology, Poznan University of Medical Sciences, Poznan, Poland

3 Department of Anatomy, Poznan University of Medical Sciences, Poznan, Poland

4 Faculty of Public Health, Mieszko I School of Pedagogy and Administartion in Poznan, Poznan, Poland

References

[1] Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature. 1983; 305(5936): 707–709.

[2] Michelmann HW, Nayudu P. Cryopreservation of human embryos. Cell Tissue Banking. 2006; 7: 135–141.

[3] Nagashima H, Kashiwazaki N, Ashman RJ, Grupen CG, Seamark MF, Nottle MB. Removal of cytoplasmic lipid enhances the tolerance of porcine embryos to chilling. Biol Reprod. 1994; 51(4): 618–622.

[4] Ghetler Y, Skutelsky E, Ben Nun I, Ben Dor L, Amihai D, Shalqi R. Human oocyte cryopreservation and the fate of cortical granules. Fertil Steril. 2006; 86(1):210–216.

[5] Stachecki JJ, Wiladsen SS. Spindle organization after cryopreservation of mouse, human and bovine oocytes. Reprod Biomed Online. 2004; 8(6): 664–672.

[6] Konc J, Kanyó K, Kriston R, Somoskői B, Cseh S. Cryopreservation of embryos and oocytes in human assisted reproduction. Biomed Res Int 2014; 2014: ID307268, 1–9.

[7] Liebermann J, Nawroth F, Isachenko V, Isachenko E, Rahimi G, Trucker MJ. Potential importance of vitrification in reproductive medicine. Biol Reprod. 2002; 67(6): 1671–1670.

[8] Jin B, Higashiyama R, Nakata Y, Yonezawa J, Xu S, Miyake M, Takahashi S, Kikuchi K, Yazawa K, Mizobuchi S, Niimi S, Kitayama M, Koshimoto C, Matsukawa K, Kasai M, Edashige K. Rapid movement of water and cryoprotectants in pig expanded blastocysts via channel processes: its relevance to their higher tolerance to cryopreservation. Biol Reprod. 2013; 89: 87.

[9] Pegg DE. The role of vitrification techniques of cryopreservation in reproductive medicine. Hum Fert. 2005; 8: 231–239.

[10] Vajta G. Are programmable freezers still needed in the embryo laboratory? Review on vitrification. Reprod BioMed Online. 2006; 6: 779–796.

[11] Fadini R, Brambillasca F, Renzini M, Merola M, Comi R, De Ponti E, Dal Canto M. Human oocyte cryopreservation: comparison between slow and ultrarapid methods. Reprod BioMed Online. 2009; 19(2): 171–180.

[12] Žáková J, Lousová E, Ventruba P, Crha I, Pochopová H, Vinklárková J, Tesařová E, Nussir M.: Sperm cryopreservation before testicular cancer treatment and its subsequent utilization for the treatment of infertility. Sci World J 2014; 2014: ID575978, 1–5.

[13] Coticchio G, Bromfield JJ, Sciajno R, Gambardella A, Scaravelli G, Borini A, Albertini DF. Vitrification may increase the rate of chromosome misalignment in the metaphase II spindle of human mature oocytes. Reprod BioMed Online. 2009; 19(3): 29–34.

[14] Saragusty J, Arav A. Current progress in oocyte and embryo cryopreservation by slow freezing and vitrification. Reproduction. 2011; 141: 1–19.

[15] Sirard MA. Factors affecting oocyte and embryo transcriptomes. Reprod Domest Anim. 2012; 4: 148–155.

[16] Wang H, Racowsky C, Combelles CMH. Is it best to cryopreserve human cumulus-free immature oocytes before or after in *vitro* maturation? Cryobiology. 2012; 65(2):79–87.

[17] Huser M, Crha I, Hudecek R, Ventruba P, Zakova J, Smardova L, Kral Z. Ovarian tissue cryopreservation—new opportunity to preserve fertility in female cancer patients. Eur J Gynaec Oncol. 2007; 28(4): 249–256.

[18] Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, Martinez-Madrid B, Van Langendonckt A. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004; 364 (9443): 1405–1410.

[19] Zakova J, Sedlackova M, Polak S, Dumkova J, Ventruba P, Crha I. Methods for preserving fertility in young women suffering from cancer: some aspects of ovarian tissue cryopreservation. Bratisl Lek Listy. 2012; 113(3): 192–194.

[20] Schroder AK, Banz C, Katalinic A, Al-Hasani S, Weiss JM, Dietrich K, Ludwig M. Counselling on cryopreservation of pronucleated oocytes. Reprod BioMed Online. 2003; 6(1):69–74.

[21] Li L, Zhang X, Zhao L, Xia X, Wang W. Comparison of DNA apoptosis in mouse and human blastocysts after vitrification and slow freezing. Mol Reprod Dev. 2012; 79: 229–236.

[22] Hwang IS, Hara H, Chung HJ, Hirabayashi M, Hochi S. Rescue of vitrified-warmed bovine oocytes with rho-associated coiled-coil kinase inhibitor. Biol Reprod. 2013; 89(2): 26.

[23] Li X, Krawetz R, Liu S, Meng G, Rancourt DE. ROCK inhibitor improves survival of cryopreserved serum/feeder-free single human embryonic stem cells. Hum Reprod. 2009; 24: 580–589.

[24] Hochi S, Abdalla H, Hara H, Shimoda M, Morita H, Kuwayama M, Hirabayashi M. Stimulatory effect of Rho-associated coiled-coil kinase (ROCK) inhibitor on revivability of *in vitro*-produced bovine blastocysts after vitrification. Theriogenology. 2010; 73: 1139–1145.

[25] Arav A, Yavi S, Zeron Y, Natan D, Dekel I, Gacitua H. New trends in gamete's cryopreservation. Mol Cell Endocrin. 2002; 187(1–2): 77–81.

[26] Huang ChCh, Lee TH, Chen SU, Chen HH, Cheng TCh, Liu ChH, Yang YS, Lee MS. Successful pregnancy following blastocyst cryopreservation using super-cooling ultrarapid vitrification. Hum Reprod. 2005; 20(1): 122–128.

[27] Kaarniranta K, Elo M, Sironen R, Lammi MJ, Goldring MB, Eriksson JE, Sistonen L, Hekminen HJ. Hsp70 accumulation in chondrocystic cell exposed to high continuous hydrostatic pressure coincides with mRNA stabilization rather than transcriptional activation. PNAS. 1998; 95: 2319–2324.

[28] Pribenszky C, Molnar M, Cseh S, Solti S. Improving post-thaw survival of cryopreserved mouse blastocyst by hydrostatic pressure challenge. Anim Reprod Sci. 2005; 87: 143–150.

[29] Houseini SM, Forouzanfar M, Hajian M, Asgari V, Abedi P, Hosseini L, Ostadhosseini S, Moulavi F, Safahani Langrroodi M, Sadeghi H, Bahramian H, Eghbalsaied Sh, Nasr-Esfahani MH. Antioxidant supplementation of culture medium embryo development

and/or after vitrification-warming; which is the most important? J Assist Reprod Genet. 2009; 26: 355–364.

[30] Hwang IS, Hochi S. Recent progress in cryopreservation of bovine oocytes. BioMed Res Int. 2014; Article ID 570647.

[31] Shapiro BS, Daneshmand ST, Restrepo H, Garner FC, Aguirre M, Hudson C. Matched-cohort comparison of single-embryo transfers in fresh and frozen-thawed embryo transfer cycles. Fertil Steril. 2012; 99: 389–392.

[32] Roque M, Lattes K, Serra S, Sola I, Geber S, Carreras R, Checa MA. Fresh embryo transfer versus frozen embryo transfer in *in vitro* fertilization cycles: a systematic review and meta-analysis. Fertil Steril. 2013; 99(1): 156–162.

[33] Schattman GL. Cryopreservation of oocytes. N Eng J Med. 2015; 373:1755–1760.