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Ovarian Tissue Cryopreservation and Transplantation: It is a Noble Goal for Mammalian Species?: A Review

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ABSTRACT

Ovarian tissue cryopreservation and transplantation is a noble goal for humanity and preserving the genetic materials of superior animal species. Females facing chemotherapy and radiation treatment are exposed to gonadal damage. In addition, superior animals due to sudden death or aging are also at risk to lose their genetic materials. Ovarian tissue cryopreservation and transplantation has been successfully carried out in different mammalian species resulting in formation of ovarian structures and outcomes upon pregnancy. Trials were applied over ovarian cryopreservation and re-transplantation in humans and different animal species. Several factors effect on ovarian cryopreservation and re-transplantation including technical aspects of freezing ovarian tissue, size of ovarian tissue, age of female, location of transplantation, auto- and xenotransplantation. Restoration of ovarian follicle growth and development, ovulation and corpora lutea formation and development were confirmed in several studies. The growth and development of ovarian follicles to pre-ovulatory follicles and corpora lutea in mammals lasts several months. In addition, *in vitro* maturation of oocytes aspirated from transplanted ovarian tissues were confirmed in addition to their further embryonic development. Therefore, this review is carried out to collect and discuss the ovarian tissue cryopreservation and transplantation and its effects on both humans and animals as well.

Key words: Cryopreservation, Development, Embryo, Maturation, Oocyte, Ovarian transplantation.

Ovarian cortex tissue egg-producing portion can be cryopreserved and transplanted later to make pregnancy possible in different mammalian species (Andersen *et al.,* 2012; Mohammed 2018a, 2019). Cryopreservation of ovarian tissue is mainly used in girls and women facing a gonadotoxic treatment for fertility preservation. Transplanted ovarian tissues regain ovarian functions in almost all patients (Yding Andersen *et al.,* 2019). Ovarian tissues' transplantation and cryopreservation offer fascinating opportunities for mammalian species (Aljubran *et al.*, 2023; Mohammed 2019). The auto-grafting and xeno-grafting of ovarian cortex tissue demonstrate potential advancement of cyopreservation (Aljubran *et al.*, 2023; Mohammed 2019). The cryopreserved ovarian tissue has been transplanted in different animal species and humans resulting in the birth of normal young [Demirci *et al.*, 2003, Donnez *et al.*, 2004). Such techniques of ovarian tissue transplantation and cryopreservation in females who suffer from cancer present the elixir of life through ovariectomy and cryopreservation before chemotherapy and radiation treatments followed by re-transplantation after recovery. Therefore, ovarian tissue cryopreservation and transplantation is a noble goal for humanity and preserving the genetic materials of superior animal species. Premature ovarian failure upon autotransplantation of cryopreserved ovarian tissue in women exposed to chemotherapy (Schmidt *et al.,* 2012).

Modulation of gonadal tissues is essential for conservation of species, increase productivity, fertility treatments of mammalian species (Ali *et al.*, 2021; Al Masruri *et al.,* 2022; Mohammed and Al-Suweigh 2023; Al Mufarji *et al.*, 2023). Various treatments have been recently applied for enhancing the purpose of ovarian transplantation (Table 1).

They include treatments for enhancement of blood vascularization, inhibition of ovarian follicle degeneration in addition to stimulation of ovarian follicle growth and development (Mohammed 2019; Oktay and Marin 2023). In addition, ovarian cryopreservation and transplantation processes and the resulting outcomes in addition to the factors effecting cryopreservation and transplantation are indicated in the following figure (Fig 1).

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Furthermore, cumulus-enclosed germinal vesicle oocytes could be aspirated from the transplanted ovarian tissues and used for *in vitro* embryo production (Mohammed *et al.,* 2005; Sánchez *et al.,* 2015; Báez *et al.,* 2022). Ovarian tissue cryopreservation and transplantation results in a 76.0% spontaneous pregnancy live baby rate in humans (Silber *et al.*, 2022). Therefore, the current review was carried out to collect, consolidate and highlight the ovarian tissue cryopreservation and transplantation.

The current review was designed according to the procedure approved by Scientific Research Deanship, King Faisal University of Saudi Arabi from November to March 2024. The articles concerning ovarian tissue collection and processing, ovarian tissues cryopreservation, ovarian transplantation process, the assisted reproductive techniques used after ovarian transplantation were collected form PubMed, science direct and google scholar in addition to our articles related to ovarian transplantation for writing the current review.

Ovarian tissue collection and processing

Ovarian tissue collection for transplantation involves removing a small piece of ovarian tissue through laparoscopy procedure (Beckmann *et al.,* 2016; Fabbri *et al.,* 2022). The removed ovaries are divided into small pieces and either transplanted or cryopreserved. The cryopreserved ovarian tissues can be stored for several years in liquid nitrogen tank (-196.0°C) especially storage facilities where samples are carefully monitored and preserved.

Practical aspects of freezing ovarian tissue

Ovarian tissue cryopreservation (OTC) has developed from being an experimental procedure to being a standard method of fertility preservation since the late 1990s. The necessitate of ovarian tissue cryopresevation includes the urgent need for chemotherapy, which indicates no time for ovarian superovulation and egg aspiration protocol (Macklon 2020; Shapira *et al.*, 2020). Fifty percent of the women had ovarian tissue transplantation achieved pregnancies and 28.4-42.0% of them delivered an infant (Shapira *et al.*, 2020; Oktay *et al.*, 2015).

Transportation of ovarian tissues prior to fresh transplantation or freezing

The transportation standard procedure of ovarian tissue is necessitated for both human and animals. The OT

Table 1: Factors effecting on ovarian tissue cryopreservation and transplantation.

Treatments	Effects	References
Size of ovarian transplanted tissue pieces	The sizes of ovarian tissue pieces should be large enough to be transplanted flat on the underlying surface and prevent its movement	Meirow *et al.*, 2005 Yding Andersen *et al.,* 2019 Lunding *et al.,* 2019
Age of transplanted ovaries	Significant increase in numbers of secondary and graffian follicles and corpora lutea of young ovaries compared to adult ones	Mohammed 2018a, 2019
Revascularizing	Ovarian functions restored	Oktay *et al.*, 2022
Genetic diseases	1- The diseases that require bone marrow transplantation is konwn to destroy ovarian function 2- The women with disorders that themselves pose a risk on follicular depletion. Transplantation has not yet been performed in this group.	Mamsen *et al.,* 2018, 2019

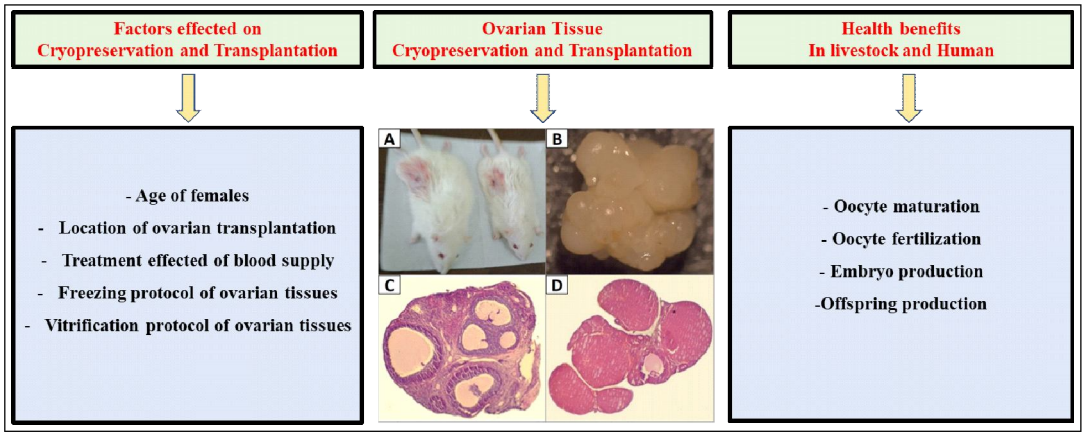

Fig 1: Ovarian cryopreservation and transplantation processes and the resulting outcomes in addition to the factors effecting cryopreservation and transplantation.

transportation procedure has been shown to be valuable in clinical practices (Kyono *et al.,* 2017). The transportation time should be minimized as much as possible. The ideal temperature for transporting ovarian tissues is around 4°C to minimize ischemic damage caused by a lack of blood flow. Ovarian tissues are transported in sterile containers designed to maintain the cool temperature. These containers typically contain a transport medium as buffer solution.

In human patients, the procedure is required where the ovarian tissue is surgically removed and other lab performing the cryopreservation (Duncan *et al.,* 2016, Kyono *et al.,* 2017). In animals, the circumstances that need ovarian tissue transportation (OTT) might include sudden or accidental death of superior animals in addition to OTT form slaughterhouses. Loss of ovarian follicles during transportation is minimal as recently shown where follicle survival of frozen and thawed tissues transported was similar to that of fresh and frozen tissues (Kristensen *et al.,* 2018).

Ovarian tissues cryopreservation

Ovarian tissues cryopreservation has been performed since more than two decades. Several technical aspects for freezing ovarian tissue should be taken into consideration including the method of freezing, the type of cryoprotectants, the size of the ovarian tissue. The most common cryoprotectants are ethylene glycol, DMSO and propylene glycol (Jadoul *et al.,* 2017; Gook, 2017). These molecules are capable of penetrating cell membrane and are always combined with a sucrose that facilitates a further reduction in intracellular water activity (Jain and Paulson, 2006). The cryoprotectants may be used either through freezing or vitrification process and discussed in many papers (Suzuki *et al.,* 2015).

It is so difficult to evaluate the efficiency of cryopreservation procedures through fertility potential upon OT or grafting as ovarian restoring function (follicle morphology, hormone production and gene expression), children born from the procedure and the duration of ovarian tissue function following transplantation. Dying *in vitro* test through vital dye neutral red that evaluates follicles survival following freezing and thawing ovarian tissues was evaluated (Kristensen *et al.,* 2018). Living follicles were shown in intense red staining whereas nonviable follicles remained unstained (Morewood *et al.,* 2017, Kristensen *et al.,* 2018). The benefit of OTC instead of follicle survival could be confirmed through tissue transplantation. Therefore, the procedures to improve ovarian tissue transplantation include enhancement of revascularization, supply of oxygen and nutrients and reduce apoptosis.

The ovarian tissue freezing protocol includes dissecting ovarian tissue into small pieces, exposing the tissues to a solution containing cryoprotectants (*e.g*. glycerol, dimethyl sulfoxide and ethylene glycol) in a stepwise manner, the tissues are subjected to a controlled cooling curve process using a programmable freezer and the frozen tissues are stored in liquid nitrogen tank (-196°C) (Isachenko *et al.,* 2012; Rivas Leonel *et al.,* 2019). There are different cooling curves as -2°C/min to -8°C, manual seeding, "0.3°C/min to -40°C and -30°C/min to -140°C (Donnez *et al.,* 2004). The vitrification is a rapid freezing technique includes dissecting ovarian tissue into small pieces, exposing the tissues to multiple solutions with increasing cryoprotectant concentration (Seki and Mazur, 2008). The freezed or vitrified ovarian tissues are stored in liquid nitrogen for long-term preservation (Rivas Leonel *et al.,* 2019).

Small versus large pieces of ovarian tissues

Restoration of ovarian activity upon transplantation are indicated through ovarian follicle development, ovulation, embryo development and children born (Fig 2). The sizes of the transplanted ovarian cortical tissues are a factor effect on the total number of surviving follicles following transplantation. The too small pieces (< 1-3 mm^3) complicate their positioning and if they are left free they may move as the woman becomes physically active. This leads to disruption of revascularization and reduce follicle survival. The large ovarian cortical tissue (around 5.0 × 5.0 × 1.0 mm) are easy to position and fix in the right place. This accelerate revascularisation, nutrients and oxygen supply leading to enhancement of follicles survival (Meirow *et al.*, 2005).

Social reasons for ovarian tissue cryopreservation

Many well-educated women in many western countries are delaying childbearing, preserving their fertility potential by

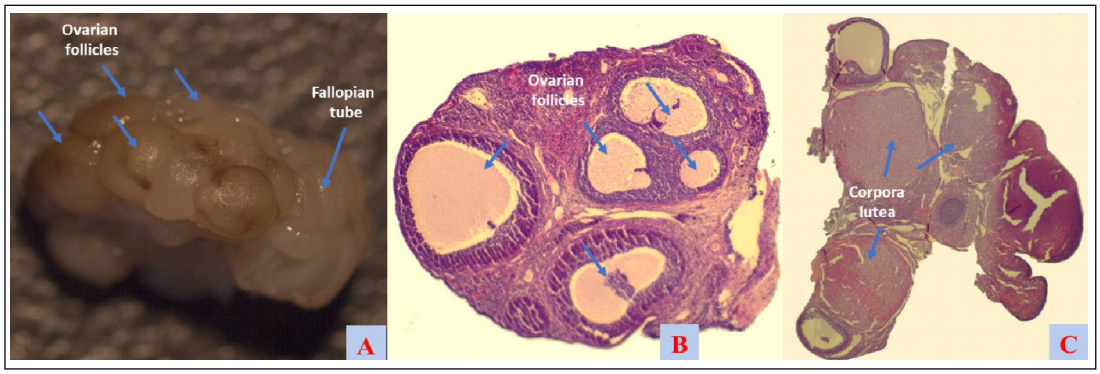

Fig 2: Ovarian tissues over transplantation in rat. A, Active transplanted ovary containing follicles (blue arrows); B, antral follicles; C, corpora lutea (Mohammed *et al.,* 2022).

freezing good quality oocytes to find the right partner and focus on their careers. The usage rate of stored oocytes range 3.0-9.0% (Ben-Rafael 2018), which indicates the need for alternative solution represented by OTC and transplantation. Such OTC and grafting procedure is a scenario for fertility and endocrine purposes. This unproven suggestion needs further investigation concerning the sizes of excised ovarian tissues and its side effect on the residual ovarian reserve. In the future, women undergoing C-section parturition may want to have a small ovarian biopsy and have it frozen for future use.

In vitro maturation of oocytes upon ovarian transplantation

In vitro maturation (IVM) of human oocytes last 24 h whereas IVM of ruminant oocytes, rodent oocytes, pig and camel oocytes last 24 h, 17 h and 42-48 h, respectively (Gordon 2003; Mohammed *et al.*, 2005, 2008, 2010, 2019a, 2022). *In vitro* maturation of oocytes is the most important step for further embryonic development (Mohammed *et al.*, 2005, 2022). Nuclear and cytoplasmic changes occur in oocytes for successful fertilization and early embryonic development (Mohammed *et al.,* 2005). Hjorth *et al.* (2020) investigated reproductive outcomes after *in vitro* fertilization upon transplantation of cryopreserved ovarian tissue. The conclusion of such study that the women of advanced age have a poor ovarian reserve, resulting in a poor reproductive outcome. Several factors effect on efficiency of oocyte maturation and further embryonic development as species, follicle sizes, oestrous or menstrual stage, age, maturation media and supplements (Mohammed 2014a,b; Mohammed *et al.,* 2008, 2010, 2019b, 2020, 2022). The quality of oocytes, cumulus cells inclusion, follicular fluid or fetal calf supplementation effected on oocyte maturation rates and embryo cleavage and blastocyst and hatched rate (Mohammed *et al.,* 2005, 2022). Additionally, nutrition and feed additives have been shown to stimulate ovarian follicles and embryo development (Mohammed 2018b; Mohammed and Attaai 2011; Mohammed *et al.,* 2012a,b, 2019b, 2020, 2023).

CONCLUSION

Ovarian tissue cryopreservation and transplantation is required for agriculture and medical applications in both animals and humans, respectively. The applications extend to *in vitro* embryo production through oocyte maturation, fertilization and embryo culture. Therefore, ovarian tissue cryopreservation and transplantation is an outstanding approach over the world for sustainable milk and meat production from ruminant animals and avoid sterility in humans due to chemotherapy and radiation treatments. Hence, the continuous development of such techniques is necessitated to become precise and maximizing embryonic development. There are several advantages of ovarian tissue cryopreservation over oocyte for patients include no delay for treatment of cancer, avoidance of ovarian superovulation and resumption of endocrine functions.

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Conflict of interest

There is no conflict of interest for authors to declare.

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