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REVIEW ARTICLE

Fertility preservation through gonadal cryopreservation

Lalitha Devi[1] · **Sandeep Goel**[1]

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Abstract Fertility preservation is an area of immense interest in today's society. The most effective and established means of fertility preservation is cryopreservation of gametes (sperm and oocytes) and embryos. Gonadal cryopreservation is yet another means for fertility preservation, especially if the gonadal function is threatened by premature menopause, gonadotoxic cancer treatment, surgical castration, or diseases. It can also aid in the preservation of germplasm of animals that die before attaining sexual maturity. This is especially of significance for valuable, rare, and endangered animals whose population is affected by high neonatal/juvenile mortality because of diseases, poor management practices, or inbreeding depression. Establishing genome resource banks to conserve the genetic status of wild animals will provide a critical interface between ex-situ and in-situ conservation strategies. Cryopreservation of gonads effectively lengthens the genetic lifespan of individuals in a breeding program even after their death and contributes towards germplasm conservation of prized animals. Although the studies on domestic animals are quite promising, there are limitations for developing cryopreservation strategies in wild animals. In this review, we discuss different options for gonadal tissue cryopreservation with respect to humans and to laboratory, domestic, and wild animals. This review also covers recent developments in gonadal tissue cryopreservation and transplantation, providing a systematic view and the advances in the field with the possibility for its application in fertility preservation and for the conservation of germplasm in domestic and wild species.

Keywords Conservation biology · Ovary · Testis · Transplantation · Xenografting

Introduction

Cryopreservation is the process of storage of viable biological samples in a frozen state to preserve them over an extended period of time. Germplasm cryopreservation has become a vital tool for the conservation of threatened and endangered species [1]. Recent advancements in the techniques of intracytoplasmic sperm injection (ICSI), and isolation and preservation of germ cells and their in-vitro and in-vivo maturation have led to a renewed interest in research related to gonadal tissue cryopreservation. In humans, testicular tissue cryopreservation offers hope for the preservation of fertility in pre-pubertal boys with cancer before exposure to gonadotoxic treatments or after surgical castration [2]. Similarly, cryopreservation of ovarian tissue is the only option to preserve fertility in pre-pubertal girls and women in whom gonadal function is threatened by premature menopause, cancer therapy, or pathological conditions [3]. Cryopreservation of gonads can also be used for the preservation of genetic potential of valuable, rare, and endangered animals whose population is affected by high neonatal/juvenile mortality because of diseases or poor management practices or inbreeding depression [4–6]. There is a growing interest in understanding the underlying fundamental aspects of cryobiology to develop more efficient cryopreservation methods [7]. The earlier slow-cooling process has been replaced by

✉ Sandeep Goel
sandeep@ccmb.res.in

[1] Laboratory for the Conservation of Endangered Species, Centre for Cellular and Molecular Biology, Council for Scientific and Industrial Research, Uppal Road, Hyderabad 500 007, India

simpler and more efficient cryopreservation approaches—such as vitrification [1]. Further to these technological advances in cryobiology, various strategies have been developed that allow long-term storage of gametes and embryos [8].

Current research is focused on newer technologies for optimal isolation and preservation of the earliest stage of male and female germ cells and their subsequent maturation. To produce fertilization-competent haploid gametes from these early-stage germ cells, methods are needed that can support their maturation and development. Gonadal tissue transplantation is one such method. In males, testis tissue transplantation provides a robust in-vivo method for studying testicular maturation, spermatogenesis, and can be used for the production of mature gametes [9]. Similarly, ovarian tissue transplantation provides a possibility for understanding the mechanism of follicular development and ovarian function in females [10]. However, as immediate transplantation of fresh tissues is not always possible or desirable, preservation of donor tissue for future use is critical. Gonadal cryopreservation in conjunction with transplantation is a feasible option for germplasm conservation in animals [11]. In humans, for cancer patients who need immediate chemotherapy, ovarian cortex and testicular tissue cryopreservation offer a promising technique for fertility preservation [12].

In this review, we discuss different techniques of gonadal tissue cryopreservation and their outcomes in animals and humans. This article also presents a systematic review of recent developments in gonadal tissue cryopreservation and transplantation that can have potential application to fertility preservation in humans and conservation of germplasm in animals.

Implications of gonadal tissue cryopreservation

In humans

Cryopreservation and transplantation of ovarian and testicular tissue have been practiced for more than a century, mainly for experimental purposes. However, now it is considered a potential strategy for preserving fertility in young patients, including children, undergoing treatment for cancer and other diseases that cause sterilization. Indications for cryopreservation of ovarian tissue include chemo- or radiotherapy in young patients, inflammatory disorders, pelvic diseases, and chromosomal abnormalities like mosaic Turners syndrome [13]. Cryobanking of gonadal tissues and cells retrieved before the initiation of cancer therapy is an attractive strategy for preserving the fertility of young and adults of both sexes. However, it is challenging to study the methodology of cryopreservation in humans because of limited access to gonadal tissue. None of the animal species studied so far have proven to be an appropriate model to study cryopreservation [14]. Nevertheless, since 1996, functional tissue has been retrieved following the thawing of cryopreserved human ovarian tissue [15]. Follicles in slices of cryopreserved-thawed ovarian tissue have been reported to successfully survive long-term organ culture and transplantation [15–17]. In 2004, autologous transplantation of frozen–thawed ovarian tissue led to the successful birth of the first human baby [18] and since then, 37 children have been born to date using this technique.

Testicular tissue cryopreservation is the only potential procedure for preserving the fertility of pre-pubertal males, allowing preservation of different testicular cells, maintaining spermatogonial stem cells in their "niche", and providing cell-to-cell contacts between somatic and germ cells. The cryopreserved immature testicular tissue can be later used for various assisted reproductive technologies (ART) [19]. The developmental stage of the testis determines the success of cryopreservation as immature testis differs from adult testis in its tissue texture and future developmental potential [20]. In adult males, cryopreserved testicular tissues are a source for sperm. Previous literature reports cryopreservation of human testicular tissue retrieved from patients with non-obstructive azoospermia [21, 22] and the subsequent use of the testicular sperm extracted from such tissue for ART [23]. Although cryopreservation of semen and spermatozoa is a well-developed technique routinely used in infertility clinics, there is still an immense scope for improvement [24]. Transplantation of cryopreserved fetal testicular tissue under the skin has been observed to increase serum testosterone levels, which leads to improvement in sperm quality and also the general somatic condition of the patient [25]. In-vitro hormone production from cryopreserved pre-pubertal testicular tissue is also reported [26].

In livestock and endangered animals

The genetic diversity of livestock and wild animals has declined over last few decades due to changing market demands and intensification of agriculture [27]. It is in the interest of the international community to conserve livestock genomes and to maintain wildlife biodiversity, as the absence of even a single species can have a huge impact on the functioning of global ecosystems [28]. Considerable funds have been allocated by several countries to establish genome resources banks for safeguarding scientifically, economically, and ecologically important plants and animals [29]. Ex-situ conservation programs focus on cryopreservation of gametes, embryos, and somatic cells as well as testis and ovarian tissues of animals. These

cryopreserved genetic resource banks prolong the genetic lifespan of animals following their death, providing a critical interface between ex-situ and in-situ conservation strategies [30]. Although significant progress has been made in cryopreservation of semen, oocytes, and embryos of several domestic species, a standardized procedure is yet to be established. In addition, maintenance of the functional competency of sperm in cryopreserved semen following insemination into a female tract remains a challenge [31].

Cryopreserved sperms are used for artificial insemination and oocytes and embryos for embryo transfer technology in the livestock industry [1]. Gamete preservation may assist in the development, protection, and distribution of domestic animals and restoration of endangered species. Long-term storage and utilization of cryopreserved germplasm also help in prolonging the length of population generation and allow higher levels of genetic variation to be maintained in smaller populations [32]. Cryopreservation and transplantation of gonads promote the improvement of reproductive efficiency and are potential means for the conservation of endangered wildlife species. However, except for our recent study on the Indian mouse deer (*Moschiola indica*) [33], there are no other reports available on the transplantation of cryopreserved testes of wild or endangered species.

Methods of gonadal tissue cryopreservation

Slow freezing

Slow freezing is a conventional method for testicular tissue cryopreservation. Slow freezing uses an optimal cooling rate specific to a given cell to avoid the production of intracellular ice crystals. However, it is a challenge to optimize conductivity to achieve a uniform cooling of the organ during the slow-freezing process [34]. There are two popular strategies of slow freezing for cryopreservation of tissues; controlled slow freezing (CSF) and uncontrolled slow freezing (USF). CSF uses programmable freezers to control variations in temperature for freezing tissues. In the CSF method, tissues at 20–25 °C are cooled at a freezing rate of 1–3 °C/min with 25 min of equilibration until the temperature reaches 3 °C. The tissues are then frozen at a rate of 1–3 °C/min to −30 °C, and further to −60 °C at a cooling rate of 30–50 °C/min. Finally, the tissues are plunged and stored in liquid nitrogen at −196 °C. In USF, tissue pieces are equilibrated in a comparatively low concentration of cryoprotectant and then frozen gradually at approximately 1 °C/min in a −80 °C freezer overnight before plunging and storing in liquid nitrogen [35]. To date, ovarian tissue has been cryopreserved only by CSF or vitrification. The current standard procedure for ovarian tissue cryopreservation is CSF [36]. CSF has been more promising than vitrification for human ovarian tissues due to the higher developmental potential of retrieved oocytes [37, 38]. This is further proven clinically with the occurrence of pregnancies and live births. Studies comparing vitrification with slow freezing have conflicting results [39]. Using CSF and ovarian transplantation, the single graft method has resulted in four pregnancies [two after in-vitro fertilization (IVF) and two spontaneous conceptions] in one patient [40] and three consecutive live births in others [41]. The first live birth from frozen to thawed ovarian cortex after orthotopic autotransplantation was reported by a Belgian group [18]. To date, slow freezing has resulted in 36 documented live births worldwide after orthotopic autotransplantation [42–44].

In animals, ovarian tissue cryopreservation and transplantation were first practiced in rodents during the 1950s [45–47]. Parrott demonstrated the restoration of fertility after orthotopic grafting of frozen–thawed ovarian tissue in mice [48]. Similar reports have been published on rats [49] and rabbits [50]. The gradual decrease in the number of wild and domestic species in the past few decades has shifted the focus on ovarian tissue preservation. Gosden et al. reported live births after autografting frozen–thawed ovarian tissue in sheep [51]. Promising results were obtained after cryopreservation of ovarian tissue from domestic and several wild species using conventional freezing protocols [52]. In domestic species, live births have been reported in sheep [51, 53–55] and Japanese quail [56] using slow-freezing protocols for ovarian tissues. Reports on the cryopreservation of ovarian tissue of wild animals are scant and show only partial success [57–60]. The technique of cryopreserving ovarian tissue avoids several practical limitations encountered in obtaining and cryopreserving fully mature oocytes from wild mammals. Results from studies on ovarian cryopreservation and transplantation are presented in Table 1. The table also provides detailed information on the survival of follicles, their morphology, and ultra-structure after thawing; follicular growth after in-vitro culture; the recovery of reproductive and endocrine function and antral follicle formation after xenotransplantation; mature oocyte formation after auto-transplantation and xeno-transplantation; blastocyst formation after auto-transplantation and in-vitro maturation/IVF-ICSI followed by embryo culture; and live births after auto-transplantation and mating.

In humans, preservation of testicular tissue is pursued for the preservation of fertility in pre-pubertal boys due for cancer chemotherapy and infertile men. Testicular tissues from boys and men have been cryopreserved either as a cell suspension or small testicular pieces. CSF of testicular

Table 1 Achievements in ovarian cryopreservation

Species	Part of ovary used	Method of cryopreservation	Application/analysis post-thaw	Main outcome	Live birth
Human [14–16, 37, 85, 94, 112–121, 163–177]	Cortical tissues [14–16, 37, 85, 94, 112–121, 163–177]	CSF [14–16, 37, 85, 112–118, 120, 163, 168, 173, 174, 176, 177] Vitrification [14, 37, 85, 94, 119, 164–168, 170–173]	Orthotopic autotransplantation [114–120, 163] Xenografting and histological analysis of grafts [16, 164, 174] Viability assessment [16, 85, 94, 173] IVC of tissues for 5 [37], 10 [168], 14 [173],16 [37] and 21 [176] days Histological analysis [15, 37, 168, 169, 171, 176] Ultra-structural analysis [165, 170, 171] Morphological assessment [14, 85, 166, 172] TUNEL analysis [167–169] Immunohistochemistry with Caspase3 antibody [172] Auto-transplantation [112, 113, 175, 177]	Survival and maturation of primordial follicles resulted in live birth [163] Preserved follicular morphology [14, 164, 166, 168, 173] Follicular survival [16, 37, 85, 94, 174, 176] Degeneration of antral follicles [37] Preservation of morphology of frozen tissue [15, 85, 165, 172] No difference in apoptosis between fresh and frozen tissues [167] Normal ultra-structure [169–171] Abnormalities in primary follicles [171] No apoptosis in primary and primordial follicles [172] FSH levels maintained and resulted in normal pregnancy [114, 115, 117–120, 175] Restoration of endocrine activity [112]	Yes [113–121, 163, 175, 177]
Monkey [59, 60, 220]	Cortical pieces	CSF [59, 60] Vitrification [59, 220]	IVC of tissues for 5 days and viability analysis [59] Membrane transport properties [60, 220]	Follicular survival and growth [59] Properties affected by animal species, solution and cooling rate [60] Water transport affected by supra zero cooling conditions [220]	No
Mouse [85, 178–192]	Whole ovaries [85, 178–181, 184–186, 189–192] Isolated follicles [182, 183] Hemi-ovaries [187]	Vitrification [85, 178–182, 185, 186, 189] CSF [182, 183, 187–192]	IVC of ovary for 0, 0.5, 2 and 4 [178] h IVC for 6 [180], 10 [185] and 12 [180, 189] days Autotransplantation [178, 188, 190, 192] Ultra-structural analysis [85, 179, 182, 185, 186] Viability assessment [179, 184] Morphological assessment [179] Orthotopic autotransplantation [181, 183, 187] Heterotopic autotransplantation and IVF [85] Expression analysis for apoptosis [185]	No differences in apoptosis and FSH levels in frozen tissues [178] Isolated follicles showed higher viability [183] Normal morphology [179] Follicular survival [85, 180] Natural mating resulted in live birth [181, 183, 187, 190–192] Morphologically intact follicles [182] Decrease in follicular viability [184] Normal ultra-structure [185, 186] Restoration of endocrine activity [188] Isolated follicles after culture express maturation genes [189]	Yes [181, 183, 187, 190–192]
Rat [193–195]	Whole ovaries [193–195]	CSF [193–195] Vitrification [195]	Orthotopic autotransplantation [193, 194] Histological analysis [195]	Natural mating resulted in pregnancy [193, 194] Follicular survival [195]	Yes [193, 194]

Table 1 continued

Species	Part of ovary used	Method of cryopreservation	Application/analysis post-thaw	Main outcome	Live birth
Rabbit [50, 95, 96, 221]	Whole ovary and cortical pieces [50, 95, 96]	CSF	Vascular autotransplantation and ultra-structural analysis [95] Autotransplantation and mating [50] IVM/IVF–ICSI/EC [96]	Recovery of ovarian function and follicular survival [50] Normal ultra-structure [95] Pregnancy and live birth [50] Blastocyst development [96]	Yes [50]
Pig [84, 197]	Whole ovaries [197], cortical pieces [84]	CSF [84, 197] Vitrification [84]	Ultra-structural [84, 197], histological analysis [84]	Cryopreserved tissues maintained normal morphology [84, 197] Ultra-structural damages evident [84]	No
Goat [99, 101, 151, 152, 198–201]	Cortical pieces [99, 151, 152, 198–201], isolated follicles [101]	CSF and vitrification [99, 101]	IVC of tissues for 24 h [99, 101, 151, 152] and 5 days Autotransplantation [198] Ultra-structural analysis of pre-antral follicles [199, 200] Cryoprotectant perfusion study [201] Histological analysis [99]	Follicles maintained viability [101] and growth [151, 152] Complete follicular development and recovery of ovarian function [198] Normal ultra-structure [199, 200] Quantification of the amount of cryoprotectants in the ovarian tissue [201] Normal morphology and follicular survival [99]	No
Sheep [50, 51, 53–55, 93, 98, 150, 202–212]	Whole ovaries [53, 202–204] Ovarian strips [53, 93, 202–204, 211, 212] Hemi-ovaries [54, 55, 93] Cortical pieces [50, 51, 98, 150, 205–208] Isolated follicles [150, 209, 210]	CSF [50, 51, 54, 55, 98, 150, 205–208, 210] Vitrification [93, 98, 211]	Vascular autotransplantation [53, 202–204] and mating [53, 202, 203] Autotransplantation and natural mating [50, 51, 54, 55, 93, 207] Ultra-structural analysis [205, 208, 210] IVC for 5 days [150, 209, 210] Cryoprotectant perfusion study [206] Isolation of COCs and IVM [98] Histological analysis [211, 212] Progesterone analysis and Vascular autotransplantation [212]	Recovery of ovarian function and follicular survival in cryopreserved transplanted ovary and ovarian strips [150, 202, 209, 210] Mature oocytes were observed [98, 203, 204] Embryo development [203] Long term ovarian function [204] Production of live donor-derived offspring from cryopreserved ovarian tissue [51, 53] Recovery of ovarian function and production of offspring after mating [54, 55, 93] Normal ultra-structure [205] Natural mating resulted in pregnancy [50] Ultra-structural damage [208] Quantification of the amount of cryoprotectants in the ovarian tissue [206] Long-term ovarian cryopreservation [207] Maintained follicular viability, however, with ovarian artery rupture [211] Recovery of endocrine function however with complete loss of follicles [212]	Yes [51, 53–55, 93]

Table 1 continued

Species	Part of ovary used	Method of cryopreservation	Application/analysis post-thaw	Main outcome	Live birth
Cattle [84, 94, 153, 213, 214]	Cortical pieces	CSF [84, 153, 213, 214]	Ultra-structural analysis [84, 153, 213, 214]	Normal ultra-structure [84, 153, 213, 214]	No
		Vitrification [84, 94]	IVC for 48 h [153]	Follicular survival and growth [153]	
			Histological analysis [84, 94]	Normal morphology [84, 94]	
				Ultra-structural damage [84]	
Dog [97]	Cortical pieces	Vitrification	Xenotransplantation	Follicular survival	No
Cat [135, 215, 216]	Cortical tissue [135, 216]	CSF	Ultra-structural analysis [216]	Normal ultra-structure [216]	No
			Xenotransplantation [135]	Development of antral follicles [135]	
	Isolated follicles [215]		IVC of isolated follicles for 1 week [215]	Follicular survival [215]	
Horse [217]	Cortical pieces	CSF	Membrane transport properties	Change in water transport properties following supra zero cooling conditions	No
Elephant [57]	Cortical pieces	CSF	Xenotransplantation	Antral follicle development	No
Wallabies [58]	Whole ovary	CSF	Xenotransplantation	Antral follicle development and recovery of endocrine function	No
Wombats [138, 218, 219]	Cortical pieces	CSF	Xenotransplantation	Recovery of endocrine function [138, 218] and antral follicle development [219]	No
Dasyurids [222]	Isolated follicles	Vitrification	IVC and viability analysis	Follicular survival	No
Japanese quail [56, 196]	Hemi-ovary [56]	CSF [56]	Orthotropic autotransplantation	Production of live donor-derived offspring from cryopreserved ovaries [56, 196]	Yes
	Whole ovary [196]	Vitrification [56, 196]			

CSF controlled slow freezing, *USF* uncontrolled slow freezing, *COCs* cumulus oocyte complexes, *IVM* in-vitro maturation, *IVF* in-vitro fertilization, *IVC* in-vitro culture, *ICSI* intracytoplasmic sperm injection, *EC* embryo culture, *TUNEL* terminal deoxynucleotidyl transferase dUTP nick end labeling, *FSH* follicular stimulating hormone

Table 2 Achievements in testis tissue cryopreservation

Species	Method of cryopreservation	Application/analysis post-thaw	Main outcome	Live birth
Mouse [9, 61, 62, 65, 70, 73, 224]	USF [9, 70] CSF [61, 62, 65, 224] Vitrification [70] USF and vitrification [73]	Orthotopic allografting [62, 70] Ectopic allografting [9, 65, 224] Ectopic autografting [61] IVC of tissues [73]	Completion of spermatogenesis [65, 70, 224] Birth of live offspring from the cryopreserved and transplanted immature testicular tissue following ICSI [9, 62] and IVC of tissues [73]	Yes [9, 62, 73]
Rat [223]	CSF	Allografting and analysis of grafts [223]	Damage in Sertoli cells, no sperm production in grafted tissues	No
Human [69, 107, 141]	CSF [69, 107, 141] Vitrification [107]	Orthotopic xenografting [69, 107, 141] Ectopic xenografting [141]	Establishment of spermatogenesis with germ cells differentiating until spermatocytes	No
Rhesus monkey [78, 104]	USF [78] Vitrification [104]	Ectopic xenografting [78] Ectopic and orthotopic xenografting [104]		
Cat [76]	USF	Ectopic xenografting	Complete loss of germ cells	No
Pig [9, 103]	USF [9] Vitrification [103]	Ectopic xenografting	Completion of spermatogenesis [9] Birth of live offspring from the cryopreserved and transplanted immature testicular tissue following ICSI [103]	Yes [103]
Sheep [35]	USF and vitrification	Ectopic xenografting	Completion of spermatogenesis	No
Cattle [75]	USF	Viability assessment IVC of tissues and testosterone assay	Cryopreserved tissues maintained cell viability and secreted testosterone	No
Buffalo [74]	USF	Viability assessment, *in* IVC of tissues/isolated cells, protein expression analysis, TUNEL analysis, testosterone assay	Cryopreserved tissues maintained cell viability, lower apoptosis, expressed germ and somatic cells-specific proteins, secreted testosterone	No
Rabbit [62]	CSF	Orthotopic xenografting	Completion of spermatogenesis	No
Indian mouse deer [33]	USF	Ectopic xenografting	Establishment of spermatogenesis with germ cells differentiating until spermatocytes	No
Chicken [79]	CSF	Orthotopic allografting	Production of live offspring following intra-magnal insemination	Yes
Japanese quail [106]	Vitrification	Ectopic allografting	Production of live offspring following intra-magnal insemination	Yes

CSF controlled slow freezing, *USF* uncontrolled slow freezing, *ICSI* intracytoplasmic sperm injection, *IVC* in-vitro culture, *TUNEL* terminal deoxynucleotidyl transferase dUTP nick end labeling, *DMSO* dimethyl sulfoxide, *FBS* fetal bovine serum

tissues is based on a lengthy cryopreservation procedure requiring an expensive rate-controlled freezer and liquid nitrogen supply. At present, CSF using dimethylsulphoxide (DMSO) as a cryoprotectant is the method of choice to cryopreserve immature testicular tissue in animals like mice [61–65], rabbit [62], hamster [61], and pig [66, 67]. Successful cryopreservation of human testicular tissues following the CSF protocol has been reported [24, 26, 68, 69]. Compared with CSF, USF is a shorter process and requires a smaller, cheaper, and portable −80 °C freezer. Cryopreservation of human testicular tissues by USF protocol was first reported by Baert and colleagues. They demonstrated that the testicular tissues cryopreserved by USF could maintain cellular ultra-structure, tubular morphology, and tissue function [70]. Thereafter, USF has also proven to be a successful cryopreservation method for testicular tissue of mice [9, 70–73], buffalo [74], cattle [75], sheep [35], cat [76], pig [77], and monkey [61, 78]. Chicken testicular tissues have also been cryopreserved using the USF protocol [79]. A live birth has been reported using transplanted sperm retrieved from USF cryopreserved-thawed testicular tissue. Studies on testis

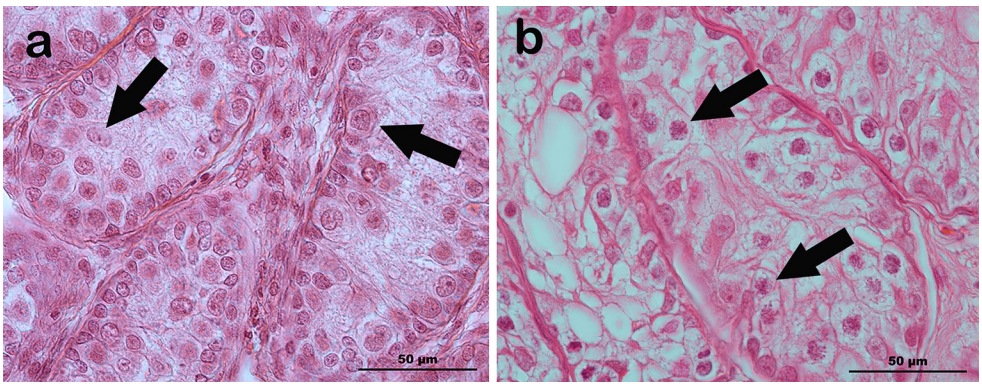

Fig. 1 Histological evaluation of cryopreserved Indian mouse deer (*Moschiola indica*) testicular tissues and xenograft with most advanced germ cell types. Typical morphology of the most advanced germ cell types in the tubule is indicated by *arrows*. **a** Donor tissue from 15-day-old Indian mouse deer showing gonocytes/spermatogonia. **b** Grafts from recipient athymic nude mice that were grafted with testicular tissues cryopreserved in cryomedium containing 10 % DMSO and 10 % FBS and collected after 6 months, which contain pachytene spermatocytes as the most advanced germ cells. *Scale bar* 50 µm

cryopreservation and transplantation and their outcomes are listed in Table 2.

Cryopreservation of gonads of wild animals is extremely challenging due to a dearth of information on the physiological and biochemical characteristics of this process. Samples are also not readily available and hence, development of efficient cryopreservation protocols is difficult. Recently, we cryopreserved an immature testis from an endangered tragulidae, the Indian mouse deer (*Moschiola indica*), using USF protocols [33]. We observed that the combinations of cryoprotectants that showed superior preservation in domestic species, monkey, human and rodents were unsuitable for the cryopreservation of mouse deer testes [33]. Only testis tissues that were cryopreserved in 10 % DMSO with 80 % fetal bovine serum (FBS) could establish spermatogenesis and induce germ cell differentiation following xenografting onto nude mice (Fig. 1). These results indicate that it is likely that optimal combination(s) of cryoprotectants differ and need to be evaluated for efficient gonadal cryopreservation of different species.

Vitrification

The solidification of liquid upon rapid cooling due to a sudden increase in viscosity is termed vitrification. Theoretically, the formation of ice crystals and their growth can be eliminated by vitrification. This process is simple as well as cost effective. Vitrification protects both intracellular and extracellular components of tissue from ice formation during cryopreservation [80]. Vitrification uses a solution with a higher osmolality than that used for slow freezing. This is followed by rapid submersion in liquid nitrogen, thereby significantly shortening the freezing time. Vitrification has been applied to the preservation of ovarian tissues of different species. Initially, vitrification of oocytes showed potential benefits in humans [81, 82]. However, further attempts to vitrify human ovarian tissue were not encouraging due to increased necrosis in frozen tissues [83, 84]. Later, a novel technique of needle immersion vitrification demonstrated improved survival of follicles in both human and murine ovarian tissue [85]. With the advancement in technology over time, vitrification has been established as a reliable strategy for the cryopreservation of oocytes [86–88]. A recent report showed no differences in fertilization, cleavage, and clinical pregnancy rates between fresh and vitrified oocytes in humans, although the ongoing pregnancy rate was reduced in the vitrified group [89]. So far in humans, only one live birth has been reported by transplanting vitrified-thawed ovarian tissues.

Domestic animals are used as model species to develop cryopreservation protocols for primordial ovarian follicles from wild species. Initial experiments in mice showed encouraging results using vitrified whole mouse ovaries [90, 91]. These vitrified-thawed ovaries showed robust preservation of oocytes with developmental competency [90] and full recovery of endocrine function [91]. In domestic species, whole-ovary cryopreservation is reported in sheep [92, 93] and cow [84, 94]. However, live birth has been reported only in sheep following the transplantation of vitrified-thawed ovarian tissue [93]. Since fewer live births were achieved after whole-ovary cryopreservation, ovarian cortex preservation is being looked at as a promising technique. Vitrification of cortical pieces is reported in rabbit [50, 95, 96], monkey [59], dog [97], cow [84, 94], sheep [98], goat [99], pig [84], and cat [100]. Isolated follicles have also been preserved by vitrification in goat to study their viability [101]. However, studies on the cryopreservation of isolated follicles are scarce because ovarian tissue cryopreservation and transplantation is less time-consuming when compared to follicle

cryopreservation, which demands specific technical skills. Recently, solid-surface vitrification (SSV) has been reported to yield better preservation of testicular tissue in pre-pubertal mouse [70] and piglet [67]. However, there is a need for improvement in the vitrification of ovarian tissue for wider clinical application and conservation of germplasm of animal species.

Encouraging results have been achieved using vitrification for the preservation of testicular tissue of mice [2, 70, 73, 102], pigs [66, 67, 103], and rhesus monkeys [104]. Felid testicular tissues showed better survival following vitrification than the laboratory rodent tissues [105]. In birds, Japanese quail testicular tissue has been vitrified successfully, resulting in live birth after transplantation [106]. However, there are limited reports on the vitrification of testicular tissues in humans [70, 107, 108]. Although vitrification is a promising technique, it is still at an infantile stage for testicular tissue cryopreservation. Therefore, further optimization of this technique is required for use in several mammalian species including humans.

Applications of cryopreserved gonadal tissues for fertility restoration

Autografting of gonadal tissues

Transplantation as a procedure for follicle maturation was first suggested by Gosden in the year 1994 [51]. Autografting of cryopreserved ovarian tissue has the potential to restore endocrine function in cancer survivors with premature ovarian failure. Ovarian tissue can be transplanted orthotopically to the pelvis [109, 110] or heterotopically to subcutaneous areas such as the forearm or abdomen [111, 112]. Regardless of the site of transplantation, potential follicular atresia due to ischemia in the ovarian grafts after transplantation remains a limitation of this technique. Nevertheless, there has been a rapid increase in the number of ovarian tissue cryopreservation and autografting procedures performed worldwide. Transplantation of frozen–thawed ovarian tissues has been successful in both laboratory and domestic animals. Live births have been reported after orthotopic transplantation of frozen–thawed ovarian tissues or whole ovary in mouse [48], rat [49], rabbit [50], and sheep [51, 53–55, 93]. In birds, live birth has been reported after orthotopic transplantation of cryopreserved ovary of Japanese quail [56].

Restoration of ovarian function after chemotherapy or radiotherapy has two main goals in humans: to improve quality of life and restore reproductive function. There are several case reports of ovarian tissue autografting for restoring fertility in humans [18, 113–120]. Donnez et al. reported the first live birth from orthotopically autografted ovarian tissue fragments in a woman who was undergoing chemotherapy for stage IV Hodgkin's lymphoma [18]. Live birth after autografting of cryopreserved pre-pubertal ovarian tissue was also reported recently [121]. To date, 37 live births have been reported following autografting of cryopreserved ovarian tissue.

Transplantation of testicular tissue provides an alternative strategy to the use of spermatogonial stem cell (SSC) suspensions. This approach maintains the SSCs within their natural niche, therefore retaining the interactions between the germ cells and their supporting somatic cells. At first glance, testis did not appear very promising for transplantation or grafting because of its tough capsule, complex vascular architecture and sensitivity to ischaemia. Testis grafting as an experimental endeavour has a long history in reproductive biology [122, 123]. Grafting of testis tissue was developed as a tool for androgen substitution in the 1950s [124] and, consequently applied to study steroidogenesis and Sertoli or Leydig cell functions [125]. Historically, autografting of frozen testicular tissue in monorchid rats was performed to understand testicular tissue function [126]. Till date, autografting of cryopreserved testicular tissues is reported only in rhesus monkey. In this study, spermatozoa were detected in the autologous-grafted cryopreserved tissues [127]. To the best of our knowledge there is no report on autografting of testicular tissues in humans.

Xenografting of gonadal tissues

Xenografting of gonadal tissues is an alternative strategy for fertility preservation in young cancer patients and endangered species. Immunodeficient mice (severe combined immunodeficiency and athymic nude) have proven to be a useful in-vivo model for the xenografting of human ovarian tissue to investigate ovarian function and follicle development. Xenografting allows for the maturation of follicles and also helps in the detection of cancer transmission and relapse in humans. The use of laboratory animals for medical research has major ethical advantages when compared to clinical trials. The development of mature (MII) oocytes from cryopreserved human ovarian cortex has been reported [128]. Xenografting of frozen ovarian tissue in mouse [129], human [130–134], cat [135], cow [136], African elephant [57], marmoset monkey [137], common wombat [138], and wallaby [58] to immunodeficient mice has resulted in antral follicle development. However, no live births have been reported in domestic or wild animal species from oocytes collected from xenografts of cryopreserved ovarian tissue to date. A study in mice demonstrated that in contrast to mature oocytes and embryos, ovarian tissues can be collected irrespective of

age or reproductive cycle and even following death [139, 140]. These findings may have a significant impact on the germplasm preservation of animals, as ovarian tissues can be collected and preserved from young females who die due to unknown etiology.

Xenografting of testicular tissues provides a lucrative process for the differentiation of germ cells into gametes without re-introducing cancer cells into a cured patient. Testicular tissue transplantation is effective in inducing spermatogenesis in a small piece of immature testis tissue. The main advantage of this method is that it is applicable to diverse mammalian species using immunodeficient mice as a host animal. Testis cryopreservation, when combined with testis tissue xenografting, can be a powerful method for the conservation of germplasm of rare and endangered species [11]. Frozen–thawed testicular tissues following xenografting onto mice showed the establishment of spermatogenesis and germ cell differentiation into spermatocytes in monkeys [78, 104], humans [69, 107, 141], cats [76], and Indian mouse deer [33], while in pigs [9, 103, 142], sheep [35], mice [61, 62, 65], and rabbits [62], the completion of spermatogenesis with production of haploid spermatids was observed. Spermatozoa retrieved from cryopreserved-xenografted immature testicular tissue have produced live offspring in mice [62], rabbits [62], and pigs [103] after ICSI. The establishment of spermatogenesis and proliferation of spermatogonia following the xenografting of cryopreserved immature testicular tissuehas been reported in humans [69, 141]. Taken together, testis tissue transplantation is a feasible option for retrieving sperm from immature testis tissues, but its efficiency varies across species.

In-vitro culture of gonadal tissues

Identification of factors that affect the regulation of ovarian function during in-vitro culture is a topic of great interest. Research is focused on understanding the in-vitro follicle growth to develop methods that support maturation of a competent egg [143]. The various approaches adopted to study follicle development include hormone production by ovaries and gamete maturation. To date, no in-vitro model has been identified that replicates a complete human ovarian cycle. The ovulation process poses a unique investigative challenge and studies are limited to in-vivo or in-vitro-perfused ovary models [144, 145]. In-vitro culture of pre-antral follicles is an attractive strategy for generating mature oocytes. Live birth after in-vitro culture of mouse primordial follicles has been reported [146]. Following encouraging results in mouse, newer methods for in-vitro oocyte maturation for human follicles were attempted. Culturing of human ovarian follicles did result in follicle activation; however, individual follicles failed to survive [147]. Nevertheless, live birth from cultured human ovarian tissue following IVF has been reported [148]. A recent report showed that exposure to an increased dose of alkylating agents prior to ovarian cryopreservation significantly lowers the survival of human ovarian follicles in culture [149]. To the best of our knowledge, there is no report so far on follicular growth in cryopreserved human ovarian tissue.

Gonads of domestic animals are used as models for developing and testing the culture methods that maybe applicable to endangered animal species. Culture of cryopreserved ovarian cortex resulted in follicular survival and growth in sheep [150], goat [151, 152], cow [153], and monkey [59]. The possibility of retrieving mature oocytes from the frozen pre-antral follicles following culture is of immense interest to researchers. Whole ovaries, cortical strips, and follicles of laboratory animals are used extensively to test the toxic effects of different cryoprotective agents and subsequent transplantation outcomes [154, 155]. A report showed that markers for early follicular development were expressed even after freezing and 48 h culture of primordial follicles in rhesus monkey [156]. These results are optimistic for the cryopreservation of ovarian tissue from human and wild animal species.

Spermatogenesis is one of the most complex processes of sequential cell proliferation and differentiation in the body. Infertility is one of the side effects of cancer therapy that has a huge psychological impact on cancer survivors [157]. Therefore, in addition to cancer treatment, fertility preservation is important for improving the psychological health of these survivors, especially young patients [158]. However, there is a possibility of reintroduction of malignant cells after autografting of testicular tissues in cancer patients. A study reported that even 20 leukemic cells, introduced into seminiferous tubules of the host, were sufficient to cause a leukemic state [159]. To avoid such adverse effect of autografting, attention was diverted towards in-vitro spermatogenesis in testicular tissues. Studies on in-vitro spermatogenesis were initiated with organ culture experiments about a century ago. The earliest report demonstrated progress of spermatogenesis up to the pachytene stage of meiosis in testis tissues of a newborn mouse placed on a clot [160]. Later, the air–liquid interphase method was developed as an organ culture system for spermatogenesis, which succeeded in obtaining functional sperm from neonatal mouse testis [161]. The extension of this method to cryopreserved testis tissues resulted in haploid gametes and birth to live pups following micro-insemination and ICSI [162]. Feasibility of the air–liquid interphase method needs to be evaluated in other species including humans before it could see widespread application.

Future prospective

Assessment of viability and quality of cryopreserved gonadal tissue is the primary requirement for the success of cryopreservation techniques. Additional insights into the prevention of cryoinjury will contribute toward the establishment of improved cryopreservation protocols for fertility preservation. The genetic abnormalities in the embryo generated from frozen to thawed gonads prior to transfer need to be studied. Although currently the success of ovarian cryopreservation is very low, strategies are being developed to preserve unfertilized oocytes and induce maturation in artificial ovaries, which can improve pregnancy rates. Further refinement of the techniques and modification of cryopreservation strategies will help in further improvements in germplasm preservation in humans and animals in the near future.

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Compliance with ethical standards

Conflict of interest Lalitha Devi and Sandeep Goel declare that they have no conflicts of interest.

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