Focus on Fertility Preservation

Animal models for fertility preservation in the male

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Abstract

Fertility preservation in the male is routinely focused on sperm. In clinical and veterinary settings, cryopreservation of sperm is a widely used tool. However, the goals for male fertility preservation differ between experimental models, maintenance of livestock, conservation of rare species, and fertility protection in men. Therefore very different approaches exist, which are adapted to the specialized needs for each discipline. Novel tools for male fertility preservation are explored targeting immature germ cells in embryonic or immature testes. Many options might be developed to combine germline preservation and generation of sperm *ex vivo* leading to interesting new perspectives. This review highlights current and future options for male fertility preservation with a special focus on animal models and a consideration of the various disciplines in need of novel tools.

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Traditional and novel options for male fertility preservation

Fertility preservation in the male usually focuses on cryopreservation of sperm as the routine technique in human and veterinary medicine (Kliesch *et al.* 1997, Li *et al.* 2007, Maxwell *et al.* 2007, Sancho *et al.* 2007, Saragusty *et al.* 2007) (Fig. 1; Table 1). This method has become the standard operating procedure, both in attending issues of male infertility in adult patients and in the routine management of livestock populations. In patients, sperm is banked before the onset of potentially gonadotoxic chemotherapeutical regimes in oncological and autoimmune diseases (Quinn & Kelly 2000, Ginsberg *et al.* 2008). In livestock management, sperm from donor breeding males presenting a series of desirable characteristics are used to artificially inseminate females in order to ensure optimum revenue from the resulting offspring. Furthermore, sperm cryopreservation is being employed in order to establish fertility reserves from endangered species, although the successful derivation of offspring is yet to be demonstrated in most species. One of the major limitations of sperm cryopreservation for male fertility preservation is the fact that mature sperm cannot be retrieved from prepubertal donors; thus, this approach is restricted to adult donor populations.

Modern strategies may open different and versatile approaches to generate male gametes with full fertilization ability. Methods currently under investigation

include the xenologous and autologous transplantation of testicular tissues and germline (stem) cells, the development of procedures to enable *in vitro* spermatogenesis, and the optimization of cryopreservation protocols for testicular cells and tissues. If tools become available to generate male gametes from immature germline precursors, fertility preservation can also focus on the maintenance of germline cells at any stage of development. In this scenario, preservation of cells is performed on precursors of sperm (e.g. spermatogonial cryopreservation). The target population is made up from any germline cell starting with primordial germ cells in the epiblast of the early embryo to spermatogonia in the adult testis. The preservation of immature germline cells is only valuable when strategies become available to differentiate immature germ cells into sperm with fertilizing ability. In this regard, various breakthrough studies have opened fascinating and promising perspectives. Figure 1 lists many approaches which can be categorized into tools providing germline preservation, fertility preservation, or both aspects. Certainly, a creative combination of the various approaches opens many potential novel pathways to facilitate long-term preservation of the male germline and generation of sperm.

In this review, we will present cryopreservation strategies using sperm from adult males and the subsequent use in assisted reproduction techniques (ART). We will pay particular attention to animal models that have attempted culture or xenotransplantation of

Figure 1 Differential and shared approaches for germline and fertility preservation.

testicular tissues and cells from a broad spectrum of donor species and age ranges to present the wide spectrum of fertility preservation in the male. Some of these studies aim at understanding the physiology of testicular stem cells and their interaction with the stem cell niches. Others attempt to re-create fully functional testicular tissues *ex situ* for the production of functional sperm.

The goal for male fertility preservation: fertilization of an oocyte

The final goal for male fertility preservation is the achievement of a pregnancy and the generation of offspring. Fertilization of an oocyte is one of the most critical steps in this process. Egg quality is a highly critical factor. Poor egg quality diminishes the chances for fertilization independent of the male gamete, because, in contrast to the sperm that primarily adds a haploid chromosome to the fertilization process, the egg adds in addition to its haploid genome critical structural and biochemical support for the early phases of embryonic development.

Oocytes cannot only be fertilized by mature and live sperm (Fig. 2). In research and in clinical settings, intracytoplasmic sperm injection (ICSI) has opened novel options for fertilization with haploid immature

germ cells that have no fertilization potential under natural or *in vitro* conditions. Immature elongating and elongated spermatids can be retrieved from testicular biopsies by testicular sperm extraction (TESE). TESE has been originally developed to retrieve sperm from testicular tissue of patients who have no sperm in the ejaculate, but show some degree of spermatogenesis in testicular biopsies. Although this strategy has quickly been implemented in infertility clinics in many countries after its original description (Palermo *et al.* 1992), it is illegal in some countries. Interestingly, this approach had never been tested in animal models prior to its human application and has – in contrast to its widespread use in ART clinics – found very limited use as an experimental tool in research and veterinary medicine.

Astounding results were achieved in mouse studies after injection of spermatocytes into eggs (Kimura & Yanagimachi 1995). The microinjected secondary spermatocytes were not only able to undergo the two meiotic divisions in the oocyte cytoplasm but were also able to provide a haploid genome for generation of normal offspring. Similarly, surprising is the finding that freeze-dried preparations of sperm have the full developmental potential to generate offspring when injected into mouse eggs (Wakayama & Yanagimachi 1998). Freeze-dried sperm must be considered dead. The integrity of the genome in these sperm was maintained for several months at room temperature. These findings indicate that the eggs have an enormous fertilization potential even when injected with immature germ cells at a tetraploid stage or a haploid genome from dead sperm. Whenever the male genome can be reconstructed, an egg can initiate the fertilization and developmental process. Other cells have been used for fertilization trials on oocytes. Although somatic cell nuclear transfer is not a natural act of fertilization, since no gametes are involved it can be regarded as an experimental approach for male fertility preservation, at least when a male somatic cell like Sertoli cell (Wakayama *et al.* 1998) is used for the transfer. In cloning a diploid nucleus is reprogrammed and the egg is stripped of its own genome. This emphasizes the great potential of eggs to generate offspring from whatever genome it was obtained from. Egg quality seems to be a major parameter for success of fertilization in both veterinary and human-assisted

Table 1 Methods of sperm collection on different species.

Species	Sperm retrieval				
	From Ejaculate			Surgical	
	Electroejaculation	From Uterus	Natural Ejaculation	Epididymal	Testicular
Rodents	✓	✓	X	✓	(✓)
Livestock	X	X	✓	(✓)	(✓)
Nonhuman primate	✓	X	✓	(✓)	(✓)
Man	(✓)	X	✓	(✓)	✓

✓, routinely performed; (✓), occasionally performed or under development; X, not performed.

Figure 2 Potential scenarios to induce fertilization.

reproduction approaches (Krisher 2004, Sirard *et al.* 2006). A controversial but highly relevant research field would be created if strategies can be developed to haploidize somatic cells and to create artificial gametes. In recent years strategies for *in vitro* haploidization have been explored, however, as yet with limited success (Tesarik & Mendoza 2003, Nagy & Chang 2007). Although highly speculative, for the future it may be possible that routes for transfer of haploidized somatic cells or *in vitro* matured artificial gametes are developed, which may include the injection of naked DNA into eggs. In addition, it has been shown that embryonic stem cells can differentiate into male gametes *in vitro* (Toyooka *et al.* 2003, Geijsen *et al.* 2004), indicating the possibility to derive functional sperm from embryonic stem cells (for review, see Daley 2007). Figure 2 depicts schematically the potential scenarios to induce fertilization.

Animal models for sperm collection and long-term preservation

In laboratory rodents, collection of sperm samples is usually performed after killing the animal. However, alternative strategies are available for collection of sperm and long-term storage opening up various pathways to explore experimentally novel options for male fertility preservation. In mice and rats, sperm can be collected either by electroejaculation (Tecirlioglu *et al.* 2002a, 2002b) or by the collection of ejaculated sperm from the uteri of females (Yamauchi & Ward 2007, Yamauchi *et al.* 2007). As indicated, the most commonly used procedure is the retrieval of sperm from the cauda epididymis of the deeply anesthetized or dead male animal (Klinefelter *et al.* 1991, Yamauchi *et al.* 2007). Long-term preservation of mouse sperm can either be achieved by standard cryopreservation protocols (Kaneko *et al.* 2006), by evaporative drying of sperm before freezing (Li *et al.*

2007) or freeze-drying procedures and maintenance of the freeze-dried sample at room temperature (Wakayama & Yanagimachi 1998).

A whole industry exists in respect to semen collection and cryostorage in farm animals. Semen collection and artificial insemination have become routine procedures in livestock reproduction. Semen is usually collected employing an artificial vagina (Curry 2007). Only under experimental conditions, the collection of epididymal sperm has been attempted (Martins *et al.* 2007). In many livestock species (e.g. cattle, horse), sperm from desired breeding males are cryopreserved, shipped, and used to inseminate large numbers of females (Morrell 2006, Haugan *et al.* 2007, Metcalf 2007, Saragusty *et al.* 2007). In some species (e.g. pig), no satisfactory cryopreservation procedure for sperm have been developed yet, so samples of fresh semen are routinely shipped for artificial insemination of sows (Gerrits *et al.* 2005).

In nonhuman primates, sperm is routinely collected by electroejaculation (Amboka & Mwenetha 2003, Leibo *et al.* 2007) and research has focused on developing adequate long-term cryopreservation methods (Morrell & Hodges 1998). On some occasions, penile vibratory stimulation has also been proposed as a less invasive procedure (Yeoman *et al.* 1998). Other authors have recently retrieved epididymal sperm for cryopreservation (Dong *et al.* 2008). Nonhuman primate sperm have been used for fertilization of oocytes employing ICSI, and live birth from the resulting pregnancies have been reported (Nusser *et al.* 2001, Ng *et al.* 2002).

Testicular tissue as a target for male fertility preservation

Compared with the widespread routine use of sperm cryopreservation and artificial insemination in both the livestock industry and the medical field, the cryopreservation of gonadal tissues for fertility preservation has as yet only entered an experimental stage. Concerning female reproductive tissues, some clinical studies involving pregnancies after autotransplantation of cryopreserved ovarian tissues (Demeestere *et al.* 2006) and the derivation of embryos *in vitro* from cryopreserved and allografted ovarian tissues (Donnez *et al.* 2007) have caught recent public attention. Regarding the successful use of germ cells from cryopreserved male reproductive tissues, clinical studies have shown success with the derivation of sperm via TESE from cryopreserved biopsies obtained from adult infertility patients (Zitzmann *et al.* 2006), but many additional approaches have been explored in animal models.

The retrieval and cryopreservation of testicular tissue has been successfully attempted in animal models, both targeting basic research and preclinical issues. This approach allows the retrieval and long-term storage of germline stem cells within their natural niche even from

adult and sexually immature donors (Paris & Schlatt 2007, Milazzo *et al.* 2008). Recently, the derivation of functional sperm has been attempted from those cryopreserved immature tissues employing a variety of *ex situ* culture approaches in animal models, indicating that these cryopreserved tissues hold the potential to produce sperm long after the testicular tissues have been removed from the immature donors (Schlatt *et al.* 2002, Jahnukainen *et al.* 2007). This approach would be of high interest to prepubertal patients where no other form of fertility preservation is possible (Wyns *et al.* 2007). Xenografting will be extremely interesting for livestock management since xenografting accelerates the production of sperm from any desired immature donor, especially in species with long periods from birth to puberty as is the case in many farm animals but also in primates where this advantage has already been described. A significant acceleration of spermatogenic induction was already shown in monkeys (Honaramooz *et al.* 2004). The acceleration of sperm production has high significance for livestock management as it could decrease the turnover time from one generation to the next. In the following, we will explore in more detail potential options for the generation of sperm from adult or immature testicular tissue.

Successful TESE has been reported from fresh testicular tissues from men and nonhuman primates (Fig. 3; Hewitson *et al.* 2002), and the retrieved sperm has been employed to produce offspring. Whereas TESE has been widely used both in the reproductive clinic and in several animal models, little use has apparently been seen in this technique in domestic animals, as studies addressing this approach in livestock species have not been reported.

Cryopreserved testicular tissue from adult donors can be maintained for indefinite periods of time. After thawing, the tissue can be used as a source for sperm. Adult murine testicular tissue can routinely be cryopreserved (Milazzo *et al.* 2008). Sperm can be retrieved from cryopreserved adult mouse testicular tissue and used for production of offspring (Ogonuki *et al.* 2006). As yet, no studies describe the successful retrieval of sperm or spermatids from cryopreserved testicular tissues in either farm animals or monkey species. Human

Figure 3 Live offspring using sperm derived from fresh or cryopreserved testicular tissues have been produced in rodents, nonhuman primates, and man, but not in livestock species.

testicular tissue, however, has been cryopreserved after retrieval of biopsies from patients with non-obstructive azoospermia (Dafopoulos *et al.* 2005a, 2005b). Sperm derived by TESE from cryopreserved biopsies was used for assisted fertilization (Zitzmann *et al.* 2006). The clinical application revealed that this strategy can in principle be applied as an experimental tool or for livestock management.

The successful extraction of mature sperm from cryopreserved adult testicular tissue could be expected in consideration of the high resistance of ejaculated sperm to cryopreservation-induced damage. It was interesting to explore experimentally whether normal sperm could also be retrieved from immature testicular tissue, which was cryopreserved and then allowed to mature *ex situ*. Many studies in rodents focused on extracting functional sperm from testicular tissue, which was dissected from immature animals and underwent sexual maturation either *in vitro* or after xenografting into a suitable host. Whereas all attempts to culture immature testicular tissues *in vitro* until sexual maturation of the seminiferous epithelium have failed so far, very promising results have been obtained by xenografting immature testicular tissues into immunodeficient mouse hosts (Fig. 4).

We showed that ectopically xenografted immature testicular tissue matures in a nude mouse host and contains differentiated germ cells up to the stage of spermatids and sperm after several weeks (Honaramooz *et al.* 2002). Mouse offspring could be generated using sperm from xenografted tissue dissected from neonatal donors (Schlatt *et al.* 2003). Others have shown that live offspring can be produced using sperm retrieved from cryopreserved and subsequently xenografted immature mouse testis tissues using TESE and *in vitro* insemination (Shinohara *et al.* 2002). Initiation of complete spermatogenesis as well as active steroidogenesis has also been shown in xenografted immature testicular tissues derived from the Djungarian Hamster (Schlatt *et al.* 2002). By contrast, as yet all attempts to xenograft adult testicular tissue have resulted in poor development and cessation of spermatogenesis (Fig. 5).

Cryopreservation and xenografting of immature testicular tissues were experimentally attempted in some farm animals, with one recent study showing the production of live offspring after xenografting of immature donor testis tissue in chicken (Song & Silversides 2007). In several other livestock species, it has been shown that haploid germ cells up to the stage of mature spermatids develop in xenografted immature testicular tissue, but no live offspring has been reported to date using xenografted sperm (Oatley *et al.* 2005, Rathil *et al.* 2006, Zeng *et al.* 2006, Kim *et al.* 2007).

We have performed preclinical studies in nonhuman primates to assess the feasibility of cryopreservation of testicular tissue as a potentially relevant clinical tool. Since xenografting of immature testicular tissues appears

Figure 4 The production of live offspring by employing sperm retrieved from xenografted immature testicular tissues has so far only been successful in a limited number of species, and has not been shown in other species (shaded grey).

much more promising compared with xenografting of adult tissue and since the target patient group for testicular tissue preservation in a clinical setting would be prepubertal patients we performed these studies using neonatal and juvenile rhesus macaques. As a prerequisite for additional studies we compared various strategies in order to optimize the cryopreservation protocol for immature rhesus monkey testicular tissue (Jahnukainen *et al.* 2007). We determined that cryomedia containing 1.4 M dimethyl sulfoxide (DMSO) showed a better outcome in terms of recovery after xenografting than testicular tissue cryopreserved with ethylene glycol or lower concentrations of DMSO. We did use a simple, uncontrolled freezing protocol that worked well in this study but might be open for additional optimization after systematic experiments are performed. We also attempted to show if nonhuman primate testicular tissues can be maintained on ice for 24 h prior to xenografting (Jahnukainen *et al.* 2007). The rationale behind these studies was to explore if a time window exists in which immature testicular tissue – like many other organs prior to transplantation – can be transported on ice prior to processing without noticeable deterioration of the tissue. If the tissue allows short-term storage on ice without significant deterioration, it could be retrieved at any location and be shipped to a centralized facility that is specialized on the more complex procedures required for testicular tissue xenografting. Here, the tissue could then be processed and xenografted under optimum conditions. Our study revealed that a storage for 24 h on

ice did not reduce the potential of immature rhesus monkey testes to survive as a xenograft and to initiate spermatogenic differentiation.

Cryopreservation of human testicular tissue has attained increasing attention as a novel approach for fertility preservation in patients prior to cancer therapy (Gosden 2002, Hovatta 2003, Orwig & Schlatt 2005, van den Berg *et al.* 2007, Keros *et al.* 2007). This strategy is also applicable to prepubertal patients whose testes are surgically removed due to cryptorchidism (Kvist *et al.* 2006). Attempts have been made to optimize the cryopreservation procedure in order to improve germ cell survival in these tissues (Keros *et al.* 2005). The xenografting procedure has been used with limited success to mature prepubertal testicular tissues for subsequent sperm extraction (Wyns *et al.* 2007).

Outlook and perspective

Cryopreservation of sperm, germ cells, and testicular tissue has become a standard operating procedure in the propagation of livestock species and in assisted reproduction clinics. In livestock management, the strategies for cryopreservation of sperm are constantly improved. Novel approaches might target species (e.g. pigs) for which currently no useful large-scale procedures for industrial use are available. In regard to the protection of endangered species, many recent studies focus on felids (Zambelli *et al.* 2006, Swanson *et al.* 2007). The intention of this work is to enable wildlife reserves and zoos the exchange of male gametes from the limited numbers of captive males. Furthermore, a cryogenic repository is established for those highly endangered species for which only few individuals remain in the wild and/or captivity.

Xenografting of testicular tissue has become a valuable experimental tool to explore testicular physiology and male germ cell development. This tool may open novel scenarios for preservation of the male germ lineage and may create useful strategies also for conservation of valuable livestock and rare animals (Paris & Schlatt 2007). Instead of focusing on preservation of mature sperm, these strategies will focus on preservation of tissue from immature donors. It is to be expected that the recent and ongoing studies on the cryopreservation of

Figure 5 Micrographs of rhesus monkey testicular tissue recovered 4 months after xenografting into nude mouse host. Note that advanced spermatogenesis is established in the tissue obtained from a juvenile rhesus monkey (a), and that no spermatogenic activity is seen in testicular tissue obtained from an adult rhesus monkey (b). Scales = 50 μ m.

nonhuman primate testicular tissues will eventually lead to novel technologies in fertility preservation, with a focus of the current studies on establishing a fertility reserve in prepubertal primates. These clinically relevant studies might also lead to new approaches for fertility preservation in boys undergoing oncological treatments or other gonadotoxic therapeutic regimens. In addition to studies in nonhuman primates, many of the technologies developed in a variety of animal models will eventually lead to safe and efficient methods to avoid long-term reproductive failure in patients undergoing gonadotoxic treatments in adult life as well as during childhood. Thus, exciting results can be expected in the near future, leading to both advances in basic reproductive sciences and to the development procedures of immense interest to the agroindustrial community, wildlife protection agency, and the field of reproductive medicine.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- Amboka JN & Mwethera PG 2003 Characterization of semen from olive baboons. *Journal of Medical Primatology* **32** 325–329.
- Van den Berg H, Repping S & van der Veen F 2007 Parental desire and acceptability of spermatogonial stem cell cryopreservation in boys with cancer. *Human Reproduction* **22** 594–597.
- Curry MR 2007 Cryopreservation of mammalian semen. *Methods in Molecular Biology* **368** 303–311.
- Dafopoulos K, Griesinger G, Schultze-Mosgau A, Orief Y, Schöpfer B, Nikolettos N, Diedrich K & Al-Hasani S 2005a Factors affecting outcome after ICSI with spermatozoa retrieved from cryopreserved testicular tissue in non-obstructive azoospermia. *Reproductive Biomedicine Online* **10** 455–460.
- Dafopoulos K, Griesinger G, Schultze-Mosgau A, Orief Y, Schöpfer B, Nikolettos N, Diedrich K & Al-Hasani S 2005b Cumulative pregnancy rate after ICSI with cryopreserved testicular tissue in non-obstructive azoospermia. *Reproductive Biomedicine Online* **10** 461–466.
- Daley GQ 2007 Gametes from embryonic stem cells: a cup half full or a cup half empty? *Science* **316** 409–410.
- Demeestere I, Simon P, Buxant F, Robin V, Fernandez SA, Centner J & Delbaere A 2006 Ovarian function and spontaneous pregnancy after combined heterotopic and orthotopic cryopreserved ovarian tissue transplantation in a patient previously treated with bone marrow transplantation: case report. *Human Reproduction* **21** 2010–2014.
- Dong Q, Rodenburg SE, Huang C & Vandevoort CA 2008 Cryopreservation of Rhesus Monkey (*Macaca mulatta*) Epididymal Spermatozoa Before and After Refrigerated Storage. *Journal of Andrology* **29** 283–292.
- Donnez J, Dolmans MM, Pirard C, Van Langendonck A, Demylle D, Jadoul P & Squifflet J 2007 Allograft of ovarian cortex between two genetically non-identical sisters: case report. *Human Reproduction* **22** 2653–2659.
- Geijsen N, Horoschak M, Kim K, Gribnau J, Eggen K & Daley GQ 2004 Derivation of embryonic germ cells and male gametes from embryonic stem cells. *Nature* **427** 148–154.
- Gerrits RJ, Lunney JK, Johnson LA, Pursel VG, Kraeling RR, Rohrer GA & Dobrinsky JR 2005 Perspectives for artificial insemination and genomics to improve global swine populations. *Theriogenology* **63** 283–299.
- Ginsberg JP, Ogle SK, Tuchman LK, Carlson CA, Reilly MM, Hobbie WL, Rourke M, Zhao H & Meadows AT 2008 Sperm banking for adolescent and young adult cancer patients: sperm quality, patient, and parent perspectives. *Pediatric Blood and Cancer* **50** 594–598.
- Gosden RG 2002 Gonadal tissue cryopreservation and transplantation. *Reproductive Biomedicine Online* **4** 64–67.
- Haugan T, Gröhn YT, Kommisrud E, Ropstad E & Reksen O 2007 Effects of sperm concentration at semen collection and storage period of frozen semen on dairy cow conception. *Animal Reproduction Science* **97** 1–11.
- Hewitson L, Martinovich C, Simerly C, Takahashi D & Schatten G 2002 Rhesus offspring produced by intracytoplasmic injection of testicular sperm and elongated spermatids. *Fertility and Sterility* **77** 794–801.
- Honaramooz A, Snedaker A, Boiani M, Schöler H, Dobrinski I & Schlatt S 2002 Sperm from neonatal mammalian testes grafted in mice. *Nature* **418** 778–781.
- Honaramooz A, Li MW, Penedo MC, Meyers S & Dobrinski I 2004 Accelerated maturation of primate testis by xenografting into mice. *Biology of Reproduction* **70** 1500–1503.
- Hovatta O 2003 Cryobiology of ovarian and testicular tissue. *Best Practice and Research. Clinical Obstetrics and Gynaecology* **17** 331–342.
- Jahnukainen K, Ehmcke J, Hergenrother SD & Schlatt S 2007 Effect of cold storage and cryopreservation of immature non-human primate testicular tissue on spermatogonial stem cell potential in xenografts. *Human Reproduction* **22** 1060–1067.
- Kaneko T, Yamamura A, Ide Y, Ogi M, Yanagita T & Nakagata N 2006 Long-term cryopreservation of mouse sperm. *Theriogenology* **66** 1098–1101.
- Keros V, Rosenlund B, Hultenby K, Aghajanova L, Levkov L & Hovatta O 2005 Optimizing cryopreservation of human testicular tissue: comparison of protocols with glycerol, propanediol and dimethylsulfoxide as cryoprotectants. *Human Reproduction* **20** 1676–1687.
- Keros V, Hultenby K, Borgström B, Fridström M, Jahnukainen K & Hovatta O 2007 Methods of cryopreservation of testicular tissue with viable spermatogonia in pre-pubertal boys undergoing gonadotoxic cancer treatment. *Human Reproduction* **22** 1384–1395.
- Kim Y, Selvaraj V, Pukazhenthi B & Travis AJ 2007 Effect of donor age on success of spermatogenesis in feline testis xenografts. *Reproduction, Fertility, and Development* **19** 1869–1876.
- Kimura Y & Yanagimachi R 1995 Development of normal mice from oocytes injected with secondary spermatocyte nuclei. *Biology of Reproduction* **53** 855–862.
- Kliesch S, Kamischke A, & Nieschlag E 1997 Cryopreservation of human semen. In *Andrology: Male Reproductive Health and Dysfunction*, Ch. 18. pp 337–355. Eds E Nieschlag & HM Behre. New York: Verlag.
- Klinefelter GR, Gray LE Jr & Suarez JD 1991 The method of sperm collection significantly influences sperm motion parameters following ethane dimethanesulphonate administration in the rat. *Reproductive Toxicology* **5** 39–44.
- Krischer RL 2004 The effect of oocyte quality on development. *Journal of Animal Sciences* **82** E14–E23.
- Kvist K, Thorup J, Byskov AG, Høyer PE, Møllgård K & Yding Andersen C 2006 Cryopreservation of intact testicular tissue from boys with cryptorchidism. *Human Reproduction* **21** 484–491.
- Leibo SP, Kubisch HM, Schramm RD, Harrison RM & VandeVoort CA 2007 Male-to-male differences in post-thaw motility of rhesus spermatozoa after cryopreservation of replicate ejaculates. *Journal of Medical Primatology* **36** 151–163.
- Li MW, Biggers JD, Elmoazzen HY, Toner M, McGinnis L & Lloyd KC 2007 Long-term storage of mouse spermatozoa after evaporative drying. *Reproduction* **133** 919–929.
- Martins CF, Rumpf R, Pereira DC & Dode MN 2007 Cryopreservation of epididymal bovine spermatozoa from dead animals and its uses *in vitro* embryo production. *Animal Reproduction Sciences* **101** 326–331.

- Maxwell WM, Parrilla I, Caballero I, Garcia E, Roca J, Martinez EA, Vazquez JM & Rath D 2007 Retained functional integrity of bull spermatozoa after double freezing and thawing using PureSperm density gradient centrifugation. *Reproduction in Domestic Animals* **42** 489–494.
- Metcalf ES 2007 The efficient use of equine cryopreserved semen. *Theriogenology* **68** 423–428.
- Milazzo JP, Vaudreuil L, Cauliez B, Gruel E, Massé L, Mousset-Siméon N, Macé B & Rives N 2008 Comparison of conditions for cryopreservation of testicular tissue from immature mice. *Human Reproduction* **23** 17–28.
- Morrell JM 2006 Update on semen technologies for animal breeding. *Reproduction in Domestic Animals* **41** 63–67.
- Morrell JM & Hodges JK 1998 Cryopreservation of non-human primate sperm: priorities for future research. *Animal Reproduction Sciences* **53** 43–63.
- Nagy ZP & Chang CC 2007 Artificial gametes. *Theriogenology* **67** 99–104.
- Ng SC, Martelli P, Liow SL, Herbert S & Oh SH 2002 Intracytoplasmic injection of frozen-thawed epididymal spermatozoa in a nonhuman primate model, the cynomolgus monkey (*Macaca fascicularis*). *Theriogenology* **58** 1385–1397.
- Nusser KD, Mitalipov S, Widmann A, Gerami-Naini B, Yeoman RR & Wolf DP 2001 Developmental competence of oocytes after ICSI in the rhesus monkey. *Human Reproduction* **16** 130–137.
- Oatley JM, Reeves JJ & McLean DJ 2005 Establishment of spermatogenesis in neonatal bovine testicular tissue following ectopic xenografting varies with donor age. *Biology of Reproduction* **72** 358–364.
- Ogonuki N, Mochida K, Miki H, Inoue K, Fray M, Iwaki T, Moriaki K, Obata Y, Morozumi K, Yanagimachi R *et al.* 2006 Spermatozoa and spermatids retrieved from frozen reproductive organs or frozen whole bodies of male mice can produce normal offspring. *PNAS* **103** 13098–13103.
- Orwig KE & Schlatt S 2005 Cryopreservation and transplantation of spermatogonia and testicular tissue for preservation of male fertility. *Journal of the National Cancer Institute Monographs* **34** 51–56.
- Palermo G, Joris H, Devroey P & Van Steirteghem AC 1992 Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* **340** 17–18.
- Paris MC & Schlatt S 2007 Ovarian and testicular tissue xenografting: its potential for germline preservation of companion animals, non-domestic and endangered species. *Reproduction, Fertility, and Development* **19** 771–782.
- Quinn B & Kelly D 2000 Sperm banking and fertility concerns: enhancing practice and the support available to men with cancer. *European Journal of Oncology Nursing* **4** 55–58.
- Rathi R, Honaramooz A, Zeng W, Turner R & Dobrinski I 2006 Germ cell development in equine testis tissue xenografted into mice. *Reproduction* **131** 1091–1098.
- Sancho S, Casas I, Ekwall H, Saravia F, Rodriguez-Martinez H, Rodriguez-Gil JE, Flores E, Pinart E, Briz M, Garcia-Gil N *et al.* 2007 Effects of cryopreservation on semen quality and the expression of sperm membrane hexose transporters in the spermatozoa of Iberian pigs. *Reproduction* **134** 111–121.
- Saragusty J, Gacitua H, Pettit MT & Arav A 2007 Directional freezing of equine semen in large volumes. *Reproduction in Domestic Animals* **42** 610–615.
- Schlatt S, Kim SS & Gosden R 2002 Spermatogenesis and steroidogenesis in mouse, hamster and monkey testicular tissue after cryopreservation and heterotopic grafting to castrated hosts. *Reproduction* **124** 339–346.
- Schlatt S, Honaramooz A, Boiani M, Schöler HR & Dobrinski I 2003 Progeny from sperm obtained after ectopic grafting of neonatal mouse testes. *Biology of Reproduction* **68** 2331–2335.
- Shinohara T, Inoue K, Ogonuki N, Kanatsu-Shinohara M, Miki H, Nakata K, Kurome M, Nagashima H, Toyokuni S, Kogishi K *et al.* 2002 Birth of offspring following transplantation of cryopreserved immature testicular pieces and *in vitro* microinsemination. *Human Reproduction* **17** 3039–3045.
- Sirard MA, Richard F, Blondin P & Robert C 2006 Contribution of the oocyte to embryo quality. *Theriogenology* **65** 126–136.
- Song Y & Silversides FG 2007 Production of offspring from cryopreserved chicken testicular tissue. *Poultry Science* **86** 1390–1396.
- Swanson WF, Magarey GM & Herrick JR 2007 Sperm cryopreservation in endangered felids: developing linkage of *in situ*-*ex situ* populations. *Society of Reproduction and Fertility* **65** 417–432.
- Tecirlioglu RT, Hayes ES & Trounson AO 2002a Semen collection from mice: electroejaculation. *Reproduction, Fertility and Development* **14** 363–371.
- Tecirlioglu RT, Lachlam-Kaplan O & Trounson AO 2002b Effects of electrical stimulation and seminal plasma on the motility of mouse sperm. *Reproduction, Fertility and Development* **14** 471–478.
- Tesarik J & Mendoza C 2003 Somatic cell haploidization: an update. *Reproductive Biomedicine Online* **6** 60–65.
- Toyooka Y, Tsunekawa N, Akasu R & Noce T 2003 Embryonic stem cells can form germ cells *in vitro*. *PNAS* **100** 11457–11462.
- Wakayama T & Yanagimachi R 1998 Development of normal mice from oocytes injected with freeze-dried spermatozoa. *Nature Biotechnology* **16** 639–641.
- Wakayama T, Perry AC, Zuccotti M, Johnson KR & Yanagimachi R 1998 Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* **394** 369–374.
- Wyns C, Curaba M, Martinez-Madrid B, Van Langendonck A, François-Xavier W & Donnez J 2007 Spermatogonial survival after cryopreservation and short-term orthotopic immature human cryptorchid testicular tissue grafting to immunodeficient mice. *Human Reproduction* **22** 1603–1611.
- Yamauchi Y & Ward MA 2007 Preservation of ejaculated mouse spermatozoa from fertile C57BL/6 and infertile Hook1/Hook1 mice collected from the uteri of mated females. *Biology of Reproduction* **76** 1002–1008.
- Yamauchi Y, Adjuk A, Riel JM & Ward MA 2007 Ejaculated and epididymal mouse spermatozoa are different in their susceptibility to nuclease-dependent DNA damage and in their nuclease activity. *Biology of Reproduction* **77** 636–647.
- Yeoman RR, Sonsken J, Gibson SV, Rizk BM & Abee CR 1998 Penile vibratory stimulation yields increased spermatozoa and accessory gland production compared with rectal electroejaculation in a neurologically intact primate (*Saimiri boliviensis*). *Human Reproduction* **13** 2527–2531.
- Zambelli D, Merlo B, Iacono E, Prati F & Belluzzi S 2006 Fertilizing ability of electro-ejaculated cryopreserved semen in the domestic cat. *Reproduction in Domestic Animals* **41** 137–141.
- Zeng W, Avelar GF, Rathi R, Franca LR & Dobrinski I 2006 The length of the spermatogenic cycle is conserved in porcine and ovine testis xenografts. *Journal of Andrology* **27** 527–533.
- Zitzmann M, Nordhoff V, von Schönfeld V, Nordsiek-Mengede A, Kliesch S, Schüring AN, Luetjens CM, Kamischke A, Cooper T, Simoni M *et al.* 2006 Elevated follicle-stimulating hormone levels and the chances for azoospermic men to become fathers after retrieval of elongated spermatids from cryopreserved testicular tissue. *Fertility and Sterility* **86** 339–347.

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