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REVIEW ARTICLE

Simple gamete preservation and artificial reproduction of mammals using micro-insemination techniques

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Abstract Assisted reproductive technology (ART) has been applied in various procedures as an effective breeding method in experimental, domestic, and wild animals, and for the treatment of human infertility. Micro-insemination techniques such as intracytoplasmic injection of spermatozoa and spermatids are now routinely used ART tools. With these techniques, even immotile and immature sperm cells can be employed as donors for producing the next generation. Gamete preservation, another ART tool, has contributed to reproductive regulation, worldwide transportation, and disease protection of animal strains, and the preserved gametes have been effectively used for the production of offspring. ART is now an indispensable tool in mammalian reproduction. This review covers the latest ART tools, with a particular emphasis on micro-insemination and gamete preservation, and discusses the future direction of mammalian artificial reproductive technology.

Keywords Animal experimentation · Assisted reproductive technologies · Freeze-drying · Intracytoplasmic sperm injection · Sperm preservation

Introduction

In 1978, the birth of the first human baby developed from an oocyte fertilized with spermatozoa in vitro was reported by Steptoe and Edwards [1]. Thereafter established as "in vitro fertilization (IVF)," this technique is now routinely used in assisted reproductive technology (ART) for infertile couples who wish to have a child. In 1951, Chang [2] and Austin [3] independently reported the occurrence of "capacitation," in which spermatozoa acquired the ability to fertilize oocytes only after incubation in the female reproductive tract for several hours. Subsequently, offspring have been obtained from rabbit oocytes fertilized in vitro with spermatozoa capacitated in the female reproductive tract [4]. Studies have also shown that capacitation could be induced artificially in vitro in defined media for hamsters [5], mice [6], rats [7, 8], and humans [1]. These reports have contributed to our understanding of fertilization events and subsequent embryonic development, and have enabled further development of ART for mammals. Various ART tools have been created, improved upon, and applied to mammalian reproduction. This review introduces the latest ART tools, focusing particularly on micro-insemination and gamete preservation.

Fertilization in vitro

IVF has been used as an effective ART tool in various mammals. The advantage of IVF is that a sufficient number of embryos can be produced using low volumes of spermatozoa, and this technique has contributed favorably to genetic resource conservation and production of offspring. IVF protocols have been established for each animal. Importantly, successful IVF requires sperm with high motility and in a suitable concentration. In the mouse, differences in motility and concentration of sperm and rates of IVF vary among individuals and strains, depending on the age of the animals [9]. Success in IVF procedures is contingent upon adequate technical skill and practical

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experience. Although IVF is one of the standard ART tools for mammalian reproduction, its success is not guaranteed for all animals.

In routine IVF, oocytes can be fertilized using both fresh and temporarily stored spermatozoa. Sperm preservation has been developed and applied to genetic resource banking for various animals [10]. Although sperm suspensions are generally cryopreserved in liquid nitrogen until their use in IVF, their tolerance to freezing varies among individual animals and strains [11–13]. Furthermore, poor technical skills in the preparation and freezing of sperm and physical stress during cryopreservation often result in impaired sperm motility [14]. While the combination of IVF and sperm preservation has been demonstrated as a suitable technique for genetic resource banking, it is only applicable for healthy animal colonies. Genetic resource banks have also been used to store sperm of animal strains showing low fertility. IVF using partial dissection of the zona pellucida with a sharp glass pipette [15–17] or laser beam [18–20] has been developed for fertilizing oocytes with spermatozoa showing low motility. However, it is not applicable to all animal species.

Injection of sperm into oocytes

Intracytoplasmic sperm injection (ICSI) has been applied as a powerful ART tool in various animals, including humans [21]. This technique involves direct injection into an oocyte of a spermatozoon drawn into a thin glass pipette attached to a micromanipulator (Fig. 1). ICSI has dramatically increased the fertility potential of spermatozoa in vitro, as fertilization of oocytes can occur even when spermatozoa are immotile [22] or immature [23]. Thus ICSI can be used to produce offspring from men with infertility due to oligozoospermia or azoospermia.

The first successful ICSI in mammals was reported by Uehara and Yanagimachi [24, 25], who demonstrated that normal pronuclei were formed in oocytes after microinjection of hamster spermatozoa. Offspring produced using ICSI have now been reported for various mammals (Table 1). Interestingly, the development of mouse ICSI [26] was first described in 1995, well after the first successful human ICSI in 1992 [27]. This can be explained by the fact that the oolemma of the mouse oocyte is easily broken during injection of spermatozoa using the conventional sharp glass pipette employed for human ICSI. This vulnerability of the mouse oocyte to damage from physical stress was overcome through the use of a piezo pulse-driven micromanipulator unit [26], which can be used to puncture the oolemma with minimum damage, significantly improving the survival of oocytes after microinjection. Micromanipulation using a piezo driver has dramatically increased the success rate of mouse ICSI procedures. Although many successful mouse studies have been published since the initial report, most have used the B6D2F1 hybrid mouse strain, which has shown high oocyte survival rates after ICSI. Transgenic and knockout strains, however, have generally been produced from inbred strains such as C57BL/6. The rates of survival of C57BL/6 oocytes after ICSI are extremely low [28], as these oocytes have very poor tolerance to the damage caused by microinjection compared with oocytes derived from hybrid strains. Further improvement of ICSI protocols using frozen oocytes [29] and K^+-rich media [30] has helped to improve the survival of C57BL/6 oocytes. Although it is time-consuming to produce multiple embryos using ICSI,

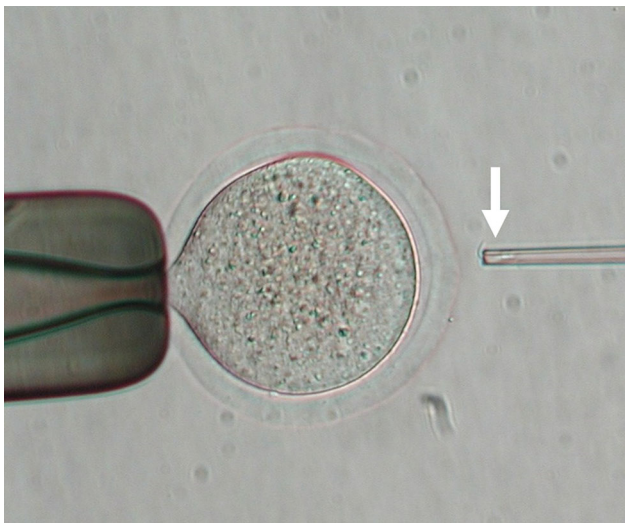

Fig. 1 ICSI in the rat. The *arrow* indicates a single sperm head with a normal shape hung on the tip of a narrow injection pipette.

Table 1 First successful full-term development of ICSI embryos in various animals

Species	Source of spermatozoa	References
Rabbit	Ejaculated	Hosoi et al. [75], Iritani and Hosoi [76]
Bovine	Ejaculated	Goto et al. [22]
Human	Ejaculated	Palermo et al. [27]
Mouse	Epididymal	Kimura and Yanagimachi [26]
Sheep	Ejaculated	Catt et al. [77]
Cat	Ejaculated	Pope et al. [78]
Horse	Ejaculated	Cochran et al. [79]
Monkey	Ejaculated	Hewitson et al. [80]
Pig	Ejaculated	Martin [81]
Rat	Epididymal	Miyata et al. [82], Hirabayashi et al. [83]
Hamster	Epididymal	Yamauchi et al. [84]
Goat	Ejaculated	Wang et al. [85]

as the process entails injecting only a single spermatozoa into each oocyte using a micromanipulator, this method is the "gold standard" for the rescue of valuable mouse strains in which conventional reproduction is difficult or impossible.

Conditions for micro-insemination

As noted above, ICSI can be used with immotile and immature spermatozoa as donor gametes to produce a new generation, and thus the motility of spermatozoa is not a factor for fertilization and preservation. Prior to the development of ICSI, testicular spermatozoa and round spermatids with no motility could not be used for in vitro fertilization of oocytes [21]. With ICSI, the ability to utilize these immature round, elongating, or elongated forms of spermatids has significantly increased the potential to produce offspring from infertile animals and humans. Offspring have been obtained even with the use of testicular spermatozoa and round spermatids collected from immature animals [31]. Historically, the success rates for producing offspring using round spermatids have been quite low, as a round spermatid lacks the ability to activate the oocyte. Artificial activation using strontium has shown some success in supporting subsequent embryonic development, and further improvements may increase its effectiveness. These fertilization techniques can be used as tools to shorten breeding cycles in the case of inbred or congenic strains, where backcrossing is normally required [32]. Successful production of offspring has also been reported with the use of oocytes injected with spermatozoa matured in vitro after collection from fresh [33] and frozen [34] testes.

Generally, spermatozoa are preserved with the aim of maintaining their motility for fertilization of oocytes in vitro. The standard method of gamete cryopreservation requires solutions containing various specialized cryoprotectants that must be stored in liquid nitrogen at −196 °C. Genetic strains of value can be preserved more simply and easily, however, with the use of ICSI. For example, offspring have been obtained from oocytes injected with immotile spermatozoa collected from the reproductive tract of specimens stored for 20 days at 4 °C [35]. Offspring have even been produced after ICSI using testicular spermatozoa collected from mouse cadavers frozen at −20 °C for 15 years, with no specialized cryopreservation [36]. These reports indicate that sperm fertility can be maintained even when spermatozoa are stored without specialized treatments for preservation. In one study, the fertilizing ability of spermatozoa was maintained in a solution adjusted to high osmolality by the addition of NaCl, without adding cryoprotectants [37]. Other studies have reported that immotile spermatozoa cryopreserved using simple culture media and Tris-based solutions without the addition of cryoprotectants were able to maintain their fertility and produce viable offspring [38–40]. Thus the development of ICSI has led to simple methods of sperm preservation and increased their fertility potential.

Simple sperm preservation by freeze-drying

Liquid nitrogen is normally used for cryopreservation of spermatozoa and other cell types, and although it is indispensable for long-term preservation of samples, the need for a continuous supply of liquid nitrogen and mechanical maintenance of the equipment used for sample storage are disadvantages. Preservation by freeze-drying has been studied as an alternative method, as it can be used to store biological materials for a prolonged period in a refrigerator (4 °C) or even at ambient temperatures (Fig. 2). After the initial report of full-term development in a mouse model [41], successful freeze-drying of spermatozoa has been described for various mammals (Table 2), and improved methods of freeze-drying have allowed spermatozoa to maintain their fertility [42]. Improvements in these methods have been well studied in mouse and rat models by Kaneko et al. [40, 43–47]. In recent studies, mouse and rat spermatozoa were preserved for 3–5 years at 4 °C after freeze-drying in a simple solution containing

Fig. 2 Freeze-dried spermatozoa in glass ampoules. The *arrow* indicates freeze-dried sperm at the bottom of the ampoules.

Table 2 Successful freeze-drying of spermatozoa from various animals

Species	Development outcome	Longest storage duration	References
Rat	Offspring	5 years	Kaneko and Serikawa [48]
Mouse	Offspring	3 years	Kaneko and Serikawa [49]
Rabbit	Offspring	More than 2 years	Liu et al. [57]
Horse	Offspring	3.5 months	Choi et al. [86]
Hamster	Offspring	1 week	Muneto and Horiuchi [58]
Bovine	Blastocysts	3 months	Keskintepe et al. [87], Martins et al. [88]
Pig	Blastocysts	1 month	Kwon et al. [89]
Monkey	8–16-cell stage	Less than 2 months	Sánchez-Partida et al. [90]

Table 3 Comparisons between cryopreservation and freeze-drying for storage of sperm samples

Contents	Cryopreservation	Freeze-drying
Storage	Liquid nitrogen tank	Refrigerator (4 °C), room temperature (short-term)
Solutions	With cryoprotectants	10 mM Tris + 1 mM EDTA (TE buffer)
Transportation	Dry-shipper (liquid nitrogen vapor), dry ice	Envelope, room temperature
Storage term in emergency	2 weeks[a]	3 months at room temperature
Backup	Liquid nitrogen tank	Refrigerator (4 °C)[b]

[a] Liquid nitrogen is vaporized at 5–10 L/day upon cessation of the liquid nitrogen supply.

[b] A backup may not be required, as samples can be stored for short periods at room temperature.

10 mM Tris buffer and 1 mM ethylenediaminetetraacetic acid (EDTA) [48, 49]. Moreover, animal generations derived from oocytes fertilized with freeze-dried spermatozoa have been shown to be healthy, with normal life expectancy and reproductive potential [48–50].

Protection of sperm DNA during preservation is an important factor for subsequent development of oocytes after ICSI. The addition of an EDTA or ethylene glycol tetraacetic acid (EGTA) chelating agent or the use of a freeze-drying solution with an alkaline pH contribute to the inactivation of internal sperm DNase [42, 43, 45]. Studies have shown that employing a Tris–EDTA buffer as a simple freeze-drying solution effectively protects against disruption of sperm DNA during storage [40, 45]. The temperature during storage of spermatozoa is also important for proper preservation. Although 4 °C is the optimal temperature for long-term preservation of freeze-dried spermatozoa, research has demonstrated they can be stored for at least three months at room temperature [47]. These findings are evidence that freeze-drying of sperm allows for easy and safe transportation worldwide and short-term preservation at ambient temperatures that requires neither liquid nitrogen nor dry ice. In fact, offspring have been obtained from oocytes fertilized with freeze-dried sperm transported by air between Japan and the United States at ambient temperatures [51]. This method clearly has many advantages over conventional cryopreservation (Table 3). Evaporation has also been used for sperm preservation as an alternative method to freeze-drying, [52, 53]. Normal offspring have been produced from oocytes injected with sperm that were dried on a glass slide using nitrogen gas. With developments in dry-state preservation such as freeze-drying and evaporation, liquid nitrogen is no longer required. As such, the concept of gamete preservation must evolve, and future innovations in dry-state oocyte and embryo preservation will further establish their efficacy. Kaneko [54] has reported detailed protocols for freeze-drying spermatozoa, as well as anticipated results.

Application of freeze-dried spermatozoa in endangered species conservation

The technique of freeze-drying of spermatozoa has now been applied in the conservation of wild animal species. Many species are endangered or threatened with extinction [55], and ART methods can be used in conservation efforts for species experiencing reproductive difficulties under both captive and wild conditions [56]. Gamete preservation, especially for spermatozoa, is an effective method for the recovery of animal populations. Because the timing of oocyte production is limited by the female reproductive cycle, it is very difficult to collect spermatozoa and oocytes at the same time for fertilization in vitro. Since spermatozoa are more easily accessible than oocytes, it is much more practical to collect the spermatozoa and preserve them temporarily. Although cryopreservation has been the standard method for storing spermatozoa, there are many animal species without established freezing and collecting protocols. Freeze-drying of spermatozoa is an important contributor to resource conservation, as the standard freeze-drying protocol can be applied for various species [48, 49, 57, 58]. Chimpanzee, giraffe, and jaguar sperm samples have been freeze-dried in the same solution (10 mM Tris and 1 mM EDTA) that is used for mouse and rat spermatozoa. Moreover, the fertilizing ability of these sperm samples can be maintained with preservation at 4 °C [59].

Gamete preservation in liquid nitrogen requires a continuous supply of liquid nitrogen and mechanical maintenance of equipment. Unfortunately, valuable sperm samples

stored using this method can be lost if the supply of liquid nitrogen is interrupted, especially during natural disasters such as earthquakes and typhoons [60]. Establishing safe facilities and equipment for use in the event of emergencies is not cost-effective. Freeze-drying sperm samples is a simple technique that can rescue many animal species from endangerment or threat of extinction, and can be envisioned as the ultimate "freeze-dried zoo" to protect against the extinction of wild species including mammals, birds, reptiles, amphibians, and fish.

Gene modification by sperm vectors using ICSI

Genome editing using engineered endonucleases, such as zinc-finger nucleases (ZFNs) [61], transcription activator-like effector nucleases (TALENs) [62–64], and the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) systems [65, 66], provides a powerful vehicle for producing genetically engineered animals. Gene knockout and knock-in animal models can be produced easily and rapidly through the use of direct injection of engineered endonucleases into oocytes, without the use of embryonic stem cells [67]. It is known that exogenous materials injected together with sperm into oocytes are integrated into chromosomes [68, 69], and such simple transgenesis via ICSI has been established for the production of genetically engineered animals. In addition, simple sperm preservation techniques such as freeze-drying can effectively aid efforts to preserve animal strains in cases where the number of strains has increased exponentially as a result of genetic engineering.

Conclusions

Here, we have reviewed the latest ART tools, with a particular emphasis on micro-insemination and gamete preservation. The development of new fertilization methods such as ICSI using micromanipulation has dramatically increased the fertility potential of spermatozoa. Furthermore, simple preservation of spermatozoa that requires neither specialized cryoprotectants nor liquid nitrogen is now possible. With the use of freeze-drying, sperm samples can be preserved over a long period of time and transported safely and easily, without the need for liquid nitrogen. Surprisingly, micromanipulation has led to methods of producing new generations of animals without the use of male gametes. Cloning techniques using somatic and stem cells have had a high impact on artificial mammalian reproduction [70, 71], and cloned animals have already been produced for many species. Although the parthenogenetic development of oocytes is normally impossible in mammalian reproduction, research using gene modification has demonstrated parthenogenetic development of mouse oocytes that have formed offspring [72]. Other studies have shown that spermatozoa and oocytes can be differentiated from induced pluripotent stem cells, and offspring have been obtained from these differentiated gametes [73, 74]. It is expected that additional novel techniques for artificial reproduction will be developed in the near future.

Disclosures

Conflict of interest The author declares no conflict of interest.

Human rights and informed consent This article does not contain any studies with human subjects.

Animal studies All institutional and national guidelines for the care and use of animals were followed.

References

1. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet. 1978;2:366.
2. Chang MC. Fertilizing capacity of spermatozoa deposited into the fallopian tubes. Nature. 1951;168:697–8.
3. Austin CR. Observations on the penetration of the sperm in the mammalian egg. Aust J Sci Res B. 1951;4:581–96.
4. Chang MC. Fertilization of rabbit ova in vitro. Nature. 1959;184:466–7.
5. Yanagimachi R, Chang MC. Fertilization of hamster eggs in vitro. Nature. 1963;200:281–2.
6. Iwamatsu T, Chang MC. In vitro fertilization of mouse eggs in the presence of bovine follicular fluid. Nature. 1969;224:919–20.
7. Miyamoto H, Chang MC. In vitro fertilization of rat eggs. Nature. 1973;241:50–2.
8. Toyoda Y, Chang MC. Fertilization of rat eggs in vitro by epididymal spermatozoa and the development of eggs following transfer. J Reprod Fertil. 1974;36:9–22.
9. Byers SL, Payson SJ, Taft RA. Performance of ten inbred mouse strains following assisted reproductive technologies (ARTs). Theriogenology. 2006;65:1716–26.
10. Agca Y. Genome resource banking of biomedically important laboratory animals. Theriogenology. 2012;78:1653–65.
11. Nakagata N. Cryopreservation of mouse spermatozoa. Mamm Genome. 2000;11:572–6.
12. Landel CP. Archiving mouse strains by cryopreservation. Lab Anim NY. 2005;34:50–7.
13. Kaenko T, Yamamura A, Ide Y, Ogi M, Yanagita T, Nakagata N. Long-term cryopreservation of mouse sperm. Theriogenology. 2006;66:1098–101.
14. Critser JK, Mobraaten LE. Cryopreservation of murine spermatozoa. ILAR J. 2000;41:197–206.
15. Malter HE, Cohen J. Partial zona dissection of the human oocyte: a nontraumatic method using micromanipulation to assist zona pellucida penetration. Fertil Steril. 1989;51:139–48.
16. Nakagata N, Okamoto M, Ueda O, Suzuki H. Positive effect of partial zona-pellucida dissection on the in vitro fertilizing capacity of cryopreserved C57BL/6 J transgenic mouse spermatozoa of low motility. Biol Reprod. 1997;57:1050–5.

17. Kawase Y, Iwata T, Ueda O, Kamada N, Tachibe T, Aoki Y, Jishage K, Suzuki H. Effect of partial incision of the zona pellucida by piezo-micromanipulator for in vitro fertilization using frozen-thawed mouse spermatozoa on the developmental rate of embryos transferred at the 2-cell stage. Biol Reprod. 2002;66:381–5.
18. Kaneko T, Yanagi M, Nakashima T, Nakagata N. The improvement in fertilizing ability of cryopreserved mouse spermatozoa using laser-microdissected oocytes. Reprod Med Biol. 2006;5:249–53.
19. Anzai M, Nishiwaki M, Yanagi M, Nakashima T, Kaneko T, Taguchi Y, Tokoro M, Shin S, Mitani T, Kato H, Matsumoto K, Nakagata N, Iritani A. Application of laser-assisted zona drilling to in vitro fertilization of cryopreserved mouse oocytes with spermatozoa from a subfertile transgenic mouse. J Reprod Dev. 2006;52:601–6.
20. Kaneko T, Fukumoto K, Haruguchi Y, Kondo T, Machida H, Koga M, Nakagawa Y, Tsuchiyama S, Saiki K, Noshiba S, Nakagata N. Fertilization of C57BL/6 mouse sperm collected from cauda epididymides after preservation or transportation at 4 degrees C using laser-microdissected oocytes. Cryobiology. 2009;59:59–62.
21. Yanagimachi R. Intracytoplasmic injection of spermatozoa and spermatogenic cells: its biology and applications in humans and animals. Reprod Biomed Online. 2005;10:247–88.
22. Goto K, Kinoshita A, Takuma Y, Ogawa K. Fertilisation of bovine oocytes by the injection of immobilised, killed spermatozoa. Vet Rec. 1990;127:517–20.
23. Kimura Y, Yanagimachi R. Mouse oocytes injected with testicular spermatozoa or round spermatids can develop into normal offspring. Development. 1995;121:2397–405.
24. Uehara T, Yanagimachi R. Microsurgical injection of spermatozoa into hamster eggs with subsequent transformation of sperm nuclei into male pronuclei. Biol Reprod. 1976;15:467–70.
25. Uehara T, Yanagimachi R. Behavior of nuclei of testicular, caput and cauda epididymal spermatozoa injected into hamster eggs. Biol Reprod. 1977;16:315–21.
26. Kimura Y, Yanagimachi R. Intracytoplasmic sperm injection in the mouse. Biol Reprod. 1995;52:709–20.
27. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 1992;340:17–8.
28. Kawase Y, Iwata T, Toyoda Y, Wakayama T, Yanagimachi R, Suzuki H. Comparison of intracytoplasmic sperm injection for inbred and hybrid mice. Mol Reprod Dev. 2001;60:74–8.
29. Sakamoto W, Kaneko T, Nakagata N. Use of frozen-thawed oocytes for efficient production of normal offspring from cryopreserved mouse spermatozoa showing low fertility. Comp Med. 2005;55:136–9.
30. Kaneko T, Ohno R. Improvement in the development of oocytes from C57BL/6 mice after sperm injection. J Am Assoc Lab Anim Sci. 2011;50:33–6.
31. Miki H, Lee J, Inoue K, Ogonuki N, Noguchi Y, Mochida K, Kohda T, Nagashima H, Ishino F, Ogura A. Microinsemination with first-wave round spermatids from immature male mice. J Reprod Dev. 2004;50:131–7.
32. Ogonuki N, Inoue K, Hirose M, Miura I, Mochida K, Sato T, Mise N, Mekada K, Yoshiki A, Abe K, Kurihara H, Wakana S, Ogura A. A high-speed congenic strategy using first-wave male germ cells. PLoS One. 2009;4:e4943.
33. Sato T, Katagiri K, Gohbara A, Inoue K, Ogonuki N, Ogura A, Kubota Y, Ogawa T. In vitro production of functional sperm in cultured neonatal mouse testes. Nature. 2011;471:504–7.
34. Yokonishi T, Sato T, Komeya M, Katagiri K, Kubota Y, Nakabayashi K, Hata K, Inoue K, Ogonuki N, Ogura A, Ogawa T. Offspring production with sperm grown in vitro from cryopreserved testis tissues. Nat Commun. 2014;5:4320.
35. Kishikawa H, Tateno H, Yanagimachi R. Fertility of mouse spermatozoa retrieved from cadavers and maintained at 4 degrees C. J Reprod Fertil. 1999;116:217–22.
36. Ogonuki N, Mochida K, Miki H, Inoue K, Fray M, Iwaki T, Moriwaki K, Obata Y, Morozumi K, Yanagimachi R, Ogura A. Spermatozoa and spermatids retrieved from frozen reproductive organs or frozen whole bodies of male mice can produce normal offspring. Proc Natl Acad Sci USA. 2006;103:13098–103.
37. Van Thuan N, Wakayama S, Kishigami S, Wakayama T. New preservation method for mouse spermatozoa without freezing. Biol Reprod. 2005;72:444–50.
38. Wakayama T, Whittingham DG, Yanagimachi R. Production of normal offspring from mouse oocytes injected with spermatozoa cryopreserved with or without cryoprotection. J Reprod Fertil. 1998;112:11–7.
39. Ward MA, Kaneko T, Kusakabe H, Biggers JD, Whittingham DG, Yanagimachi R. Long-term preservation of mouse spermatozoa after freeze-drying and freezing without cryoprotection. Biol Reprod. 2003;69:2100–8.
40. Kaneko T, Kimura S, Nakagata N. Offspring derived from oocytes injected with rat sperm, frozen or freeze-dried without cryoprotection. Theriogenology. 2007;68:1017–21.
41. Wakayama T, Yanagimachi R. Development of normal mice from oocytes injected with freeze-dried spermatozoa. Nat Biotechnol. 1998;16:639–41.
42. Kusakabe H, Szczygiel MA, Whittingham DG, Yanagimachi R. Maintenance of genetic integrity in frozen and freeze-dried mouse spermatozoa. Proc Natl Acad Sci USA. 2001;98:13501–6.
43. Kaneko T, Whittingham DG, Yanagimachi R. Effect of pH value of freeze-drying solution on the chromosome integrity and developmental ability of mouse spermatozoa. Biol Reprod. 2003;68:136–9.
44. Kaneko T, Whittingham DG, Overstreet JW, Yanagimachi R. Tolerance of the mouse sperm nuclei to freeze-drying depends on their disulfide status. Biol Reprod. 2003;69:1859–62.
45. Kaneko T, Nakagata N. Improvement in the long-term stability of freeze-dried mouse spermatozoa by adding of a chelating agent. Cryobiology. 2006;53:279–82.
46. Kaneko T, Kimura S, Nakagata N. Importance of primary culture conditions for the development of rat ICSI embryos and long-term preservation of freeze-dried sperm. Cryobiology. 2009;58:293–7.
47. Kaneko T, Nakagata N. Relation between storage temperature and fertilizing ability of freeze-dried mouse spermatozoa. Comp Med. 2005;55:140–4.
48. Kaneko T, Serikawa T. Successful long-term preservation of rat sperm by freeze-drying. PLoS One. 2012;7:e35043.
49. Kaneko T, Serikawa T. Long-term preservation of freeze-dried mouse spermatozoa. Cryobiology. 2012;64:211–4.
50. Li MW, Willis BJ, Griffey SM, Spearow JL, Lloyd KC. Assessment of three generations of mice derived by ICSI using freeze-dried sperm. Zygote. 2009;17:239–51.
51. Kaneko T. Mouse molecular embryology, methods and protocols, methods in molecular biology 1092. In: Lewandoski M, editor. The latest improvements in the mouse sperm preservation. New York: Springer; 2014. p. 357–65.
52. Bhowmick S, Zhu L, McGinnis L, Lawitts J, Nath BD, Toner M, Biggers J. Desiccation tolerance of spermatozoa dried at ambient temperature: production of fetal mice. Biol Reprod. 2003;68:1779–86.
53. Biggers JD. Evaporative drying of mouse spermatozoa. Reprod Biomed Online. 2009;19:4338.
54. Kaneko T. Chromosomal mutagenesis 2nd ed., methods in molecular biology 1239. In: Shondra M, editor. Simple sperm preservation by freeze-drying for conserving animal strains. Newyork: Springer; 2015. p. 317–29.

55. Leibo SP, Songsasen N. Cryopreservation of gametes and embryos of non-domestic species. Theriogenology. 2002;57:303–26.
56. Comizzoli P, Mermillod P, Mauget R. Reproductive biotechnologies for endangered mammalian species. Reprod Nutr Dev. 2000;40:493–504.
57. Liu JL, Kusakabe H, Chang CC, Suzuki H, Schmidt DW, Julian M, Pfeffer R, Bormann CL, Tian XC, Yanagimachi R, Yang X. Freeze-dried sperm fertilization leads to full-term development in rabbits. Biol Reprod. 2004;70:1776–81.
58. Muneto T, Horiuchi T. Full-term development of hamster embryos produced by injecting freeze-dried spermatozoa into oocytes. J Mamm Ova Res. 2011;28:32–9.
59. Kaneko T, Ito H, Sakamoto H, Onuma M, Inoue-Murayama M. Sperm preservation by freeze-drying for the conservation of wild animals. PLoS One. 2014;9:e113381.
60. Dickey RP, Lu PY, Sartor BM, Dunaway HE Jr, Pyrzak R, Klumpp AM. Steps taken to protect and rescue cryopreserved embryos during hurricane Katrina. Fertil Steril. 2006;86:732–4.
61. Geurts AM, Cost GJ, Freyvert Y, Zeitler B, Miller JC, Choi VM, Jenkins SS, Wood A, Cui X, Meng X, Vincent A, Lam S, Michalkiewicz M, Schilling R, Foeckler J, Kalloway S, Weiler H, Ménoret S, Anegon I, Davis GD, Zhang L, Rebar EJ, Gregory PD, Urnov FD, Jacob HJ, Buelow R. Knockout rats via embryo microinjection of zinc-finger nucleases. Science. 2009;325:433.
62. Tesson L, Usal C, Ménoret S, Leung E, Niles BJ, Remy S, Santiago Y, Vincent AI, Meng X, Zhang L, Gregory PD, Anegon I, Cost GJ. Knockout rats generated by embryo microinjection of TALENs. Nat Biotechnol. 2011;29:695–6.
63. Mashimo T, Kaneko T, Sakuma T, Kobayashi J, Kunihiro Y, Voigt B, Yamamoto T, Serikawa T. Efficient gene targeting by TAL effector nucleases coinjected with exonucleases in zygotes. Sci Rep. 2013;3:1253.
64. Sakuma T, Ochiai H, Kaneko T, Mashimo T, Tokumasu D, Sakane Y, Suzuki K, Miyamoto T, Sakamoto N, Matsuura S, Yamamoto T. Repeating pattern of non-RVD variations in DNA-binding modules enhances TALEN activity. Sci Rep. 2013;3:3379.
65. Yang H, Wang H, Shivalila CS, Cheng AW, Shi L, Jaenisch R. One-step generation of mice carrying reporter and conditional alleles by CRISPR/Cas-mediated genome engineering. Cell. 2013;154:1370–9.
66. Yoshimi K, Kaneko T, Voigt B, Mashimo T. Allele-specific genome editing and correction of disease-associated phenotypes in rats using the CRISPR-Cas platform. Nat Comm. 2014;5:4240.
67. Kaneko T, Mashimo T. Chromosomal mutagenesis 2nd ed., methods in molecular biology 1239. In: Shondra M, editor. Creating knockout and knockin rodents using engineered endonucleases via direct embryo injection. Newyork: Springer; 2015. p. 307–15.
68. Perry AC, Wakayama T, Kishikawa H, Kasai T, Okabe M, Toyoda Y, Yanagimachi R. Mammalian transgenesis by intracytoplasmic sperm injection. Science. 1999;284:1180–3.
69. Kaneko T, Moisyadi S, Suganuma R, Hohn B, Yanagimachi R, Pelczar P. Recombinase-mediated mouse transgenesis by intracytoplasmic sperm injection. Theriogenology. 2005;64:1704–15.
70. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. Nature. 1997;385:810–3.
71. Wakayama T, Perry AC, Zuccotti M, Johnson KR, Yanagimachi R. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature. 1998;394:369–74.
72. Kono T, Obata Y, Wu Q, Niwa K, Ono Y, Yamamoto Y, Park ES, Seo JS, Ogawa H. Birth of parthenogenetic mice that can develop to adulthood. Nature. 2004;428:860–4.
73. Hayashi K, Ohta H, Kurimoto K, Aramaki S, Saitou M. Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. Cell. 2011;146:519–32.
74. Hayashi K, Ogushi S, Kurimoto K, Shimamoto S, Ohta H, Saitou M. Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. Science. 2012;338:971–5.
75. Hosoi Y, Miyake M, Utsumi K, Iritani A. In: Development of rabbit oocytes after microinjection of spermatozoa. Proceeding of the 11th international congress on animal reproduction. 1988. abstract 331.
76. Iritani A, Hosoi Y. Microfertilization by various methods in mammalian species. Prog Clin Biol Res. 1989;294:145–9.
77. Catt SL, Catt JW, Gomez MC, Maxwell WM, Evans G. Birth of a male lamb derived from an in vitro matured oocyte fertilised by intracytoplasmic injection of a single presumptive male sperm. Vet Rec. 1996;139:494–5.
78. Pope CE, Johnson CA, McRae MA, Keller GL, Dresser BL. Development of embryos produced by intracytoplasmic sperm injection of cat oocytes. Anim Reprod Sci. 1998;53:221–36.
79. Cochran R, Meintjes M, Reggio B, Hylan D, Carter J, Pinto C, Paccamonti D, Godke RA. Live foals produced from sperm-injected oocytes derived from pregnant mares. J Equine Vet Sci. 1998;18:736–40.
80. Hewitson L, Dominko T, Takahashi D, Martinovich C, Ramalho-Santos J, Sutovsky P, Fanton J, Jacob D, Monteith D, Neuringer M, Battaglia D, Simerly C, Schatten G. Unique checkpoints during the first cell cycle of fertilization after intracytoplasmic sperm injection in rhesus monkeys. Nat Med. 1999;5:431–3.
81. Martin MJ. Development of in vivo-matured porcine oocytes following intracytoplasmic sperm injection. Biol Reprod. 2000;63:109–12.
82. Miyata T, Okada H, Hashizume R, Ito M. The offspring of intracytoplasmic sperm injection in the rat. J Mamm Ova Res. 2000;17:S24.
83. Hirabayash M, Kato M, Aoto T, Sekimoto A, Ueda M, Miyoshi I, Kasai N, Hochi S. Offspring derived from intracytoplasmic injection of transgenic rat sperm. Transgenic Res. 2002;11:221–8.
84. Yamauchi Y, Yanagimachi R, Horiuchi T. Full-term development of golden hamster oocytes following intracytoplasmic sperm head injection. Biol Reprod. 2002;67:534–9.
85. Wang B, Baldassarre H, Pierson J, Cote F, Rao KM, Karatzas CN. The in vitro and in vivo development of goat embryos produced by intracytoplasmic sperm injection using tail-cut spermatozoa. Zygote. 2003;11:219–27.
86. Choi YH, Varner DD, Love CC, Hartman DL, Hinrichs K. Production of live foals via intracytoplasmic injection of lyophilized sperm and sperm extract in the horse. Reproduction. 2011;142:529–38.
87. Keskintepe L, Pacholczyk G, Machnicka A, Norris K, Curuk MA, Khan I, Brackett BG. Bovine blastocyst development from oocytes injected with freeze-dried spermatozoa. Biol Reprod. 2002;67:409–15.
88. Martins CF, Báo SN, Dode MN, Correa GA, Rumpf R. Effects of freeze-drying on cytology, ultrastructure, DNA fragmentation, and fertilizing ability of bovine sperm. Theriogenology. 2007;67:1307–15.
89. Kwon IK, Park KE, Niwa K. Activation, pronuclear formation, and development in vitro of pig oocytes following intracytoplasmic injection of freeze-dried spermatozoa. Biol Reprod. 2004;71:1430–6.
90. Sánchez-Partida LG, Simerly CR, Ramalho-Santos J. Freeze-dried primate sperm retains early reproductive potential after intracytoplasmic sperm injection. Fertil Steril. 2008;89:742–5.