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Recent advances in fertility preservation of dogs and cats

Avanços recentes na preservação de fertilidade em cães e gatos

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Abstract

Strategies to preserve fertility in dogs and cats as cryopreservation of epididymal spermatozoa, oocytes and ovarian tissue will be discussed in this review. Recent results indicate that significant progress have been achieved, but optimal freezing protocols have not been defined yet. The extreme sensitivity of sperm membranes and of oocyte and ovarian tissue structure deserves further investigations aimed at protecting male and female gametes from freezing injuries.

Keywords: cat, dog, epididymal spermatozoa, oocytes.

Resumo

Estratégias para preservar a fertilidade em cães e gatos, como a criopreservação de esperma do epidídimo, ovócitos e tecido ovariano serão discutidos nesta revisão. Resultados recentes indicam que progresso significativo tem sido alcançado, mas protocolos ótimos de congelamento ainda não foram definidos. A sensibilidade extrema de membranas do esperma e estrutura do tecido ovariano e de ovócitos merece investigação futura com o objetivo de proteger gametas macho e fêmea de danos no congelamento.

Palavras-chave: *gato, cão, esperma do epidídimo, ovócitos.*

Introduction

Significant advances in reproductive biotechnologies aimed at preserving fertility have been recently achieved in dogs and cats.

Strategies to preserve fertility are based on cryopreservation of gametes and although freezing of ejaculated spermatozoa have been widely investigated, other opportunities as cryopreservation of epididymal spermatozoa, oocytes and ovarian tissue might open new perspectives in assisted reproduction programmes.

Fertility preservation in males

Cryopreservation of epididymal spermatozoa might significantly contribute to the preservation of genetic material and to the generation of offspring of valuable males. Epididymal spermatozoa are generally obtained from isolated epididymis caudae when the male dies unexpectedly or undergoes orchiectomy for medical reasons.

The retrieval of epididymal spermatozoa may also have relevance in individuals of high genetic or emotional value that cannot mate or ejaculate semen.

We recently demonstrated in dogs that Percutaneous Epididymal Sperm Aspiration (PESA) is a feasible alternative to the retrieval of spermatozoa from isolated organs (Varesi et al., 2013). PESA, firstly developed in men (Shah, 2011), consists in the needle aspiration of epididymal spermatozoa from cauda epididymis through the scrotal skin. The results indicated that the population of epididymal spermatozoa retrieved by PESA has similar characteristics to that collected from isolated organs, although a wide variation in sperm concentration is observed among animals.

The fertilizing ability of epididymal spermatozoa has been previously demonstrated. In dogs, intrauterine insemination with frozen spermatozoa retrieved from isolated epididymis caudae resulted in the pregnancy and in the delivery of viable puppies (Marks et al., 1994, Hori et al., 2004, 2011). In cats, frozen epididymal spermatozoa have been successfully used for artificial insemination and in vitro fertilization of oocytes (for review see Luvoni, 2006).

However, the freezing procedure negatively affects motility, membrane and acrosomal integrity of canine (Hewitt et al., 2001; Ponglowhapan et al., 2006; Hori et al., 2009) and feline (Axnér et al., 2004; Luvoni, 2006; Tebet et al., 2006; Cocchia et al., 2010) epididymal spermatozoa and several attempts have been carried out to improve the resilience of gametes to cold-damages.

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Different extenders have been tested for freezing canine (Martins et al., 2012) and feline epididymal spermatozoa (Jiménez et al., 2013). The effect of different compounds supplemented to the extenders has also been investigated. For instance, a protective effect of Equex STM paste on the acrosomes of cat spermatozoa has been shown (Axnér et al., 2004), but the presence of antioxidants in the freezing extender brought variable results.

The increase of reactive oxygen species during cryopreservation and the decrease of antioxidant activity of the spermatozoa might cause the peroxidative damage to the sperm membranes and might affect DNA integrity. Our latest results showed that DNA integrity of canine epididymal spermatozoa is well preserved after cryopreservation, but other sperm characteristics as motility and acrosomal integrity are compromised by freezing. The presence of an antioxidant compound as melatonin in the extender did not show a protective effect on post-thaw sperm quality.

We previously demonstrated in cats that epididymal sperm motility was improved after thawing by the presence of another antioxidant (taurine) in the freezing extender. However, higher proportions of thawed spermatozoa exhibited acrosomal modifications when compared to that of fresh or diluted spermatozoa before freezing (Luvoni et al., 2002).

These findings indicate that an optimal freezing protocol has not been defined yet and, although cryopreservation of epididymal spermatozoa in dogs and cats brought encouraging results, further investigations aimed at protecting gametes from freezing injuries are still needed.

Fertility preservation in females

Cryopreservation of female gametes has also important potential applications in carnivores. The availability of cryostored oocytes or gonadal tissue, as reservoir of a large number of oocytes, would greatly improve assisted reproductive techniques aimed at ensuring the future survival of high genetic value individuals.

We firstly showed that feline oocytes are able to survive freezing and we observed the development in vitro after fertilization of mature oocytes slowly frozen (for review see Luvoni, 2012).

Following this demonstration, cryosurvival of feline oocytes has been further reported. Vitrification also resulted in embryo development after warming and in vitro fertilization, and the first kittens were born in 2012 after vitrification of matured oocytes, ICSI (Intracytoplasmic Sperm Injection) and transfer of derived-embryos into recipients (Pope et al., 2012).

The low efficiency of in vitro embryo production in dogs has limited the studies on oocyte cryopreservation in this species. However, it has been recently reported that canine oocytes maintained their integrity (Abe et al., 2010) and resumed meiosis in vitro (Turathum et al., 2010) after vitrification.

Cryopreservation of cat ovarian tissue followed by xenotransplantation to immunodeficient (SCID) mice has been documented (Bosch et al., 2004). After freezing and transplantation some follicles retained the ability to resume growth from early to more advanced stages.

We firstly demonstrated that feline immature oocytes retrieved from cryopreserved ovarian tissue maintain the capability of resuming meiosis after warming (Luvoni et al., 2012). The comparative cryosurvival of oocytes vitrified as isolated or enclosed in the ovarian tissue demonstrated that different procedures of vitrification preserve the viability of feline oocytes at high extent (Alves et al., 2012).

In dogs recent results showed that ovarian tissue can be successfully preserved by slow freezing protocol as follicular growth after xenotransplantation in SCID mice has been obtained (Commin et al., 2012).

These results demonstrate that cryopreserving female gametes is an attainable goal. However, the extreme sensitivity of the female gamete to chilling injury needs further investigations aimed at optimizing the efficiency of cryopreservation protocols.

Conclusions

The cryopreservation of epididymal spermatozoa, oocyte and ovarian tissue provides the opportunity to preserve fertility. Long-term storage of gametes would allow the postponing of artificial insemination or in vitro embryo production and transfer at proper time. It would also maintain genetic diversity that would otherwise be lost when an animal dies or is gonadectomized and this is particularly crucial in wild and rare species threatened by extinction or in domestic valuable breeds.

However, some of these procedures are still at an experimental level and further investigations are required to achieve constant and repeteable results.

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