ABSTRACTS

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Cryosurvival of *ex situ* and *in situ* feline oocytes

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Significant advances in feline oocytes cryopreservation have recently been achieved. The oocyte is considered more susceptible to cooling damage than embryos and spermatozoa, however recent results demonstrate that banking of cryopreserved female gametes is an attainable goal. In Felids, as well as in other mammals, oocyte cryopreservation would significantly contribute to the improvement of assisted reproductive technologies aimed to the preservation of biodiversity. Besides the advantages in animal conservation, the use of animal models, as domestic cat, provides the opportunity for further investigations of the principles of oocyte cryobiology which can help to improve current technologies applied to both humans and animals. A review of the literature regarding survival of feline oocytes cryopreserved as isolated cells (*ex situ*) or enclosed in ovarian follicles (*in situ*) is provided.

*Ex situ* oocytes

History of cryopreservation of *ex situ* feline oocytes started in 1997 with the first report about freezing tolerance of germinal vesicle (GV) stage oocytes. Results showed that GV oocytes were able to survive as, after thawing and culture, they resumed meiosis and reached the Metaphase II (MII) in vitro. However, development in vitro after fertilization was enhanced when mature oocytes (MII-stage) were slow frozen. Following these demonstrations, cryosurvival of feline oocytes has been further reported, although variable results have been obtained with cryopreservation of oocytes at different meiotic stages (1).

Studies on other species revealed that oocytes are particularly vulnerable to cold injuries. Cytoskeleton disorganization, chromosome and DNA abnormalities, spindle disintegration, plasma membrane disruption and premature cortical granule exocytosis with related hardening of the zona pellucida are the main damages observed in thawed oocytes. In feline GV oocytes, irregular distribution of cytoskeletal elements and a decrease of cumulus-oocyte gap junction mediated communications after thawing, demonstrate their sensitivity to low temperatures (2).

Vitrification in straws, OPS (Open Pulled straws) and cryoloop of *ex situ* oocytes has also been tested and embryo development after IVF of mature (1,4) and immature oocytes (3,5) has been achieved. The first pregnancy was established last year in one recipient receiving embryos derived from vitrified immature oocytes (6). The introduction on the market of innovative cryodevices for vitrification, prompted further investigations and, very recently, first kittens were born after vitrification of matured oocytes with Cryotop, ICSI and transfer of derived-embryos into recipients (7).

*In situ* oocytes

The interest toward banking of mammalian ovarian tissue has increased progressively over the last years. The preservation of functional integrity of follicles and enclosed (*in situ*) oocytes is the main goal of the cryopreservation of ovarian cortex. It has been shown that freezing of small preantral follicles collected from feline ovaries resulted in the survival of enclosed oocytes (8).

In the ovarian cortex a population of antral follicles already exists and healthy immature oocytes can be retrieved after cryopreservation and matured in vitro for IVF. We firstly demonstrated that feline immature oocytes retrieved from cryopreserved ovarian tissue (*in situ* oocytes) maintain the capability of resuming meiosis after warming (9). Recent findings on comparative cryosurvival of *ex situ* and *in situ* feline oocytes demonstrated that different procedures of vitrification (DAP 213 and Cryotop), preserve viability of oocytes at high extent, but cumulus cells are highly susceptible to vitrification and their integrity is hardly preserved. However, the capability to resume meiosis evidenced that oocytes vitrified *ex situ* or *in situ* have comparable cryotolerance (10).

CONCLUSIONS: Cryopreservation of feline oocytes started 15 years ago and the birth of kittens from vitrified oocytes has been obtained this year (7). This success shall result in a further boost to continue these studies. The objective is the development of procedures for *ex situ* and *in situ* oocytes aiming to obtain constantly high rates of cryosurvival. Preservation of rare genotypes and maintenance of a valuable source of genetic material for research applications will hopefully be the culmination of all our efforts.


