ABSTRACTS

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Successful preservation of primordial follicles from lions by slow freezing and xenotransplantation of ovarian cortex into immunodeficient mice

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OBJECTIVES AND METHODS: Assisted reproductive technique (ART) is considered as a significant tool in the conservation of endangered species. In addition to oocytes and sperm collection for artificial insemination, in-vitro-fertilization (IVF) or embryo transfer, genome resource banking is an important part of ART. Genetic resource banking is defined as the storage of gametes (sperm and oocytes) and embryos for use in breeding programs at some future occasion. This includes breeding of behaviourally or medically incompatible pairs independently of time and location. The most limiting factor of ART is the availability of mature oocytes, which represent 1% of the ovarian stock. The main follicle pool consists of primordial follicles located in the ovarian cortex. Therefore, freezing of ovarian cortex in connection with xenotransplantation is considered as an opportunity to obtain oocytes for ART in addition to in-vitro-maturation of fully grown oocytes.

The aim of the present study was to preserve as many female germ cells as possible from female lions (Panthera leo, n = 13) that underwent ovariecotomy or euthanasia for medical or management reasons. Immediately after removal, the ovaries were shipped to the lab and were processed within 6 to 24 hours. Ovaries were cut into halves and cumulus oocyte complexes (COC) were collected by slicing the medullar side, not dissecting the outer cortex layer. Good quality COCs were subjected to in-vitro-maturation and fertilization by ICSI as previously described for domestic cats (1). Then, the thin layer of ovarian cortex (approximately 200 µm thickness) was obtained and cut into equal size pieces (2 mm diameter punch) for cryopreservation by a standard slow freezing protocol (2). The cryoprotectant contained 1.5 M ethylene glycol supplemented with 0.1 M sucrose and BSA. The viability of primordial follicles was assessed by histology with serial cuttings and haematoxylin-eosin-staining before and after thawing, and after xenotransplantation under the skin of ovariectomized immunodeficient mice. The overall number and size distribution of 40 follicles per piece was determined. According to size, the follicles were classified as shrunken (< 30µm), primordial (30 - 40 µm) or growing follicles (> 41 µm).

RESULTS: Overall, 152 intact COCs were obtained from thirteen lions. The maturation rate was only 29.6 % and only 3.2 % of oocytes cleaved after ICSI. Ovarian cortex pieces (3.14 mm²) of a mature lion contained about 300 primordial follicles. Assessed by histology, the survival rate of primordial follicles after seven days of culture was about 50 %, independent of the freezing procedure. The xenotransplantation of frozen-thawed pieces indicates that survival of primordial follicles within immunodeficient mice is at least four weeks, however: no initiation of growth was measurable.

CONCLUSION: The survival of primordial follicles within the cortex can be assessed by histology before and after cryopreservation, if culture or xenotransplantation is performed. To obtain oocytes for further ART, follicular growth initiation must be established. The results on maturation and cleavage of lion oocytes indicate that the standard domestic cat protocol is not suitable for this particular felid species.


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