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Short communication

Developmental competence of domestic cat oocytes from ovaries stored at various durations at 4 °C temperature

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ABSTRACT

Temporal storage of ovaries can provide opportunity to rescue oocytes from ovaries of endangered felids. The objective of the study was to examine the effect of different storage periods (2, 24 and 48 h) of ovaries at 4 °C for maturation of cat oocytes in vitro. Ovaries were collected from 25 domestic cats at various stages of the estrous cycle by routine ovariohysterectomy following anesthesia at different local veterinary clinics, and maintained in physiological saline at 4 °C for 2, 24 or 48 h until oocytes recovery. Selected COCs were maturated at 38 °C for 48 h in four-well petri dishes, which included 500 μL modified synthetic oviduct fluid (mSOF) medium under mineral oil in a humidified 5% CO_2, 5% O_2, and 90% N_2 atmosphere incubator. After the in vitro maturation period, there were no differences between the rate of oocytes matured at MII stages in 2 and 24 h storage groups (50.7% and 48.2% respectively, $p > 0.05$). However, the same result for the 48 h group was significantly lower than the 2 and 24 h groups (28.0%, $p < 0.001$).

Our results suggest that while 2 or 24 h storage of ovaries at 4 °C does not affect the meiotic competence of oocytes in vitro, 48 h storage of ovaries decrease the results dramatically.

1. Introduction

Most of the 36 species of wild cats are classified as threatened, vulnerable or endangered due to habitat loss. In both domestic and non-domestic cat species, the biotechnologies as in vitro maturation

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and fertilization has demonstrated great potential for assisting in the conservation of endangered cat species (Pope, 2000). The domestic cat is not only a valuable model for development of in vitro techniques but may serve as a recipient of embryos from several species of small wild cats (Farstad, 2000). Temporal storage of ovaries can provide opportunities to rescue oocytes from ovaries of endangered felids, which sometimes die suddenly in the field, or to rescue ovaries that are ovariohysterectomized for medical reasons (Naoi et al., 2007). Temperature of the transport and storage medium is a major factor affecting complete oocyte maturation in animals (Yang et al., 1990; Nakao and Nakatsuji, 1992). It has been suggested that cellular autolysis could occur in ovaries during the long conventional transportation periods (Holt and Picard, 1999). Nevertheless, both animal and human oocytes are very susceptible to cold and freezing (Massip and Leibo, 2002; Smitz et al., 2004), and exposure of oocytes to cold causes irreversible disruption in the spindle fiber of the oocyte nuclear structure (Smitz et al., 2004; Woods et al., 2004). However, it has been demonstrated that unlike other species, cat oocytes have a unique ability to mature in vitro after ovary storage 24 h at 4 °C (Otoi et al., 2001). However, little information is available concerning the possibility of recovery of viable oocytes from ovaries stored at 4 °C for prolonged storage time. The objective of the study was to examine the effect of different storage periods (2, 24 and 48 h) of cat ovaries at 4 °C for maturation of oocytes in vitro.

2. Materials and methods

2.1. Collection and storing of ovaries and oocyte recovery

Ovaries were collected from 25 domestic cats at various stages of the estrous cycle by routine ovariohysterectomy following anesthesia at different local veterinary clinics, and maintained in physiological saline at 4 °C for 2 h ($n = 9$), 24 h ($n = 8$) or 48 h ($n = 8$) until oocyte recovery. Then the ovaries were sliced with a scalpel blade and rinsed by washing medium (heparin supplemented HEPES modified TCM 199; Otoi et al., 2004) at room temperature in order to obtain cumulus oocytes complexes (COCs). COCs were washed three times with modified synthetic oviduct fluid (mSOF) medium (Bolamba et al., 2002). The oocytes with large diameter, darkly pigmented ooplasm, and completely surrounded by at least one layer of cumulus cells were selected for in vitro maturation (IVM).

2.2. In vitro maturation

Modified SOF (mSOF) was used as IVM medium supplemented with 10 μg/mL follicle stimulating hormone (FSH), 10 μg/mL luteinizing hormone (LH) and antibiotics. Selected COCs were maturated at 38 °C for 48 h in four-well petri dishes (NUNC®, Denmark), which included 500 μL maturation medium under mineral oil in each well. Incubations for IVM were carried out in a humidified 5% CO_2, 5% O_2, and 90% N_2 atmosphere. For each experimental group, 20–40 COCs were separately placed in each well according to obtained oocytes number per replication.

2.3. Assessment of the nuclear stage of maturation

At the end of IVM, oocytes were transferred into 0.2% (w/v) hyaluronidase and completely denuded by gentle pipetting. Then oocytes were placed in KCl solution (0.7%, w/v) for 3–5 min at room temperature for chromatin dispersal. Oocytes were positioned on a slide as described by Hewitt and England (1998) and were fixed with acetic acid/ethanol fixative (1/3, v/v) for 1–2 days. Aceto-orcein (2% in 45% acetic acid) staining method was used to visualize the nuclear structures. The meiotic stage of each oocyte was determined under a phase contrast microscope.

2.4. Statistical analysis

The data were analyzed using SPSS 8 (Version 10.0) for Windows (MS) and Chi-Square test was applied to examine the differences between the groups.