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Superoxide dismutase and taurine supplementation improves *in vitro* blastocyst yield from poor-quality feline oocytes

Małgorzata Ochota*, Anna Pasieka, Wojciech Niżański

Faculty of Veterinary Medicine, Department of Reproduction and Clinic of Farm Animals, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland

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ABSTRACT

Blastocyst production *in vitro* seems to be crucial part of assisted reproduction techniques in feline species. However, the results of cats' oocyte maturation and embryo development are still lower than those in other species. The aim of this study was to evaluate whether the supplementation with superoxide dismutase (SOD) and taurine during maturation or culture would improve the blastocyst yield obtained from lower grades of oocytes, that are usually discarded, as not suitable for further *in vitro* purposes. To investigate the effect of antioxidants' addition, the good- and poor-quality oocytes, were cultured with the addition of 10-mmol taurine and 600 UI/mL SOD. The nuclear maturity, embryo development, and blastocyst quality were subsequently assessed. In control group, without antioxidant supplementation, significantly less poor-quality oocytes matured (42% vs. 62%) and more degenerated (35% vs. 20%), comparing to the experimental group supplemented with SOD and taurine. The amount of obtained blastocyst was much higher, when poor quality oocytes were supplemented with SOD and taurine (supplementation to IVM—4%; supplementation to IVC—5.5%; supplementation to IVM and IVC—5.9% of blastocyst), comparing to not supplemented control group (1.3%). The best blastocysts were obtained when poor oocytes had antioxidants added only during embryo culture (185 ± 13.4 blastomeres vs. 100 ± 1.5 in control). In the present study, we reported that the lower grades of oocytes can better mature and form significantly more blastocysts with better quality, when cultured with addition of SOD and taurine.

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1. Introduction

The domestic cat (*Felis catus*) is the only one, not considered to be endangered, or at risk of extinction, from all Felids species. Hence, it can successfully be used as the biomedical model for assisted reproduction techniques, that might in future be suitable for wild cat conservation. Recently, among other achievements in domestic cats assisted reproductive techniques there are, intracytoplasmic sperm injection, cloning embryos, and cryopreservation of oocytes and embryos [1–4]. However, the *in vitro* results of cats' oocyte maturation or embryo development are still lower than those in other species. Only 40% to 60% of *in vitro*–cultured cats' oocytes reach metaphase II (MII). Similarly, although 60% to 95% of *in vitro*–produced feline embryos develop to morula, very few of them achieve blastocyst stage [5,6]. The modification of culture conditions may help to overcome such developmental blocks. The successful *in vitro* culture depends on many aspects, such as gas atmosphere, culture temperature, sources of ions, carbohydrates and protein, or added growth factor [7–9]; thus, the entirely efficient media and conditions are still to be determined for feline species.

Apoptosis (programmed cell death) is vital *in vivo* for normal embryogenesis. Suboptimal culture conditions

* Corresponding author. Tel.: +48 71 3205300; fax: +48 695 533 770.
E-mail address: malgorzata.ochota@up.wroc.pl (M. Ochota).

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in vitro may promote defective apoptotic processes leading to developmental blocks or embryo death. The oxidative stress, among others, is one of the drawback factors in embryo production. Reactive oxygen species (ROS) may be metabolic side products of developing oocytes and embryos, or originate from their surroundings, especially during aerobic conditions. Although it is possible to maintain *in vitro* 5% of O_2 with 5% CO_2 and 90% N_2, the broadly used conditions are aerobic and contain 5% CO_2 in 95% of air. The 20% of O_2 in air highly exceeds the reported oviductal concentration of 5% to 8% [10]. Oocytes and embryos are able to produce some antioxidant enzymes (superoxide dismutase, glutathione, and ɣ-glutamylcysteine synthetase); so the final influence of ROS reflects the balance between their formation and elimination. During IVC, it is very difficult to assess how many ROS are actually present under certain conditions; thus to prevent damage related to oxidative stress, various antioxidants can be added to maturation or culture medium.

There are two main types of antioxidants: enzymatic (superoxide dismutase–SOD, catalase, glutathione-s-transferase, glutathione peroxidase, ascorbate oxidase, and polyphenoloxidase) and nonenzymatic (total reduced glutathione, vitamins A, E, C, carotenoids). Many of them are present in female reproductive tract, likely playing a role in oocyte maturation and embryo development. Moreover, the oviductal fluid contains free aminoacids, with taurine and hypotaurine as one of the main constituents (41% in mice) [11], which could serve as molecular scavengers preventing the accumulation of toxic substances from hydrogen peroxide and chloride reactions, and might also be helpful when added *in vitro* [12]. There was some work done on the addition of various antioxidants i.e. beta-mercaptoethanol, cysteamine, vitamin E, taurine, hypotaurine, allopurinol, and superoxide dismutase for bovine embryo culture returning varying success [13,14]. Additionally, the supplementation of free radical scavengers was proven beneficial in mouse, hamster, pig, and rabbit IVC [15–18].

The successful IVM/IVF/IVC in domestic cats is directly proportional to the quality of the obtained oocytes. Generally, the oocyte collection for IVC is based on their morphologic appearance, taking into account the regular shape and the appearance of cytoplasm (homogenously dark), together with the quality of cumulus cell layers and intact corona radiata. It has been proven that the best chances to mature, fertilize, and develop to blastocyst *in vitro*, poses those oocytes with dark ooplasm and several, complete layers of cumulus cells. In cats, oocyte collection is made without prior hormonal stimulation and as a result, most of obtained oocytes are immature and vary significantly in their quality and competence. Unfortunately, the high-quality pool accounts for approximately 15% of all recovered ovarian oocytes, while the rest still need to be discarded, as not suitable for *in vitro* purposes, unless alternative methods for their culture would be established [19,20]. Any culture changes, allowing at least some of the remaining 85% to turn into good quality of blastocysts, might be an essential step toward the conservation of wild cat species.

The aim of this study was to evaluate whether the addition of taurine and superoxide dismutase for maturation of lower quality feline oocytes and embryo culture may be beneficial for their *in vitro* development and the quality of obtained blastocysts. The chosen concentrations of added antioxidants were similar studies investigating various concentrations of SOD and taurine done in rabbits that returned very encouraging results [16].

2. Materials and methods

Unless otherwise stated, all chemicals and reagents used in this study were purchased form Sigma–Aldrich, Poland.

2.1. Oocyte collection and IVM

Ovaries were collected from sexually matured queens during routine ovariectomy or ovariohysterectomy performed at the Department of Reproduction and Farm Animal Clinic, Faculty of Veterinary Medicine in Wroclaw, Poland. Ovaries were immediately stored in transport medium (PBS supplemented with 1% of antibiotic antimycotic solution) at 4 °C and kept no longer than 24 hours until oocyte recovery. Cumulus–oocyte complexes (COCs) were released by slicing ovaries with a scalpel blade in washing medium (WM) containing medium 199 with Earle's salts, supplemented with 3 mg/mL BSA, 0.1 mg/mL cysteine, 1.4 mg/mL HEPES, 0.25 mg/mL sodium pyruvate, 0.6 mg/mL sodium lactate, 0.15 mg/mL L-glutamine, and 0.055 mg/mL gentamicin [4]. The isolated cells were classified using a dissecting microscope and allocated to three groups. Class A: immature oocytes, round with dark, almost black, regular ooplasm pigmentation with several layers of cumulus cells. Class B: round cells with uneven dark and light ooplasm with less and disrupted cumulus cell layers. Class C: irregular cells with light ooplasm and almost no cumulus cells. The latter ones were considered degenerating and incapable of *in vitro* development and immediately discarded [18,19]. Classes A and B were assigned separately to experimental groups.

Selected class A and B oocytes were placed in 400 μL of maturation medium (WM with addition of 0.025 IU/mL of LH and 0.02 UI/mL of FSH) under mineral oil and matured for 24 hours at 38.5 °C in 5% CO_2 in air with maximum humidity. After 24 hours, nuclear maturity was assessed by the presence of the first polar body (Hoechst 33342 chromatin staining).

2.2. In vitro fertilization and in vitro culture

For IVF, thawed spermatozoa isolated from cauda epididymis and frozen according to the procedure described by Niżański et al. [21] were used. After 24-hour maturation, COCs were washed twice in WM and coincubated with 1×10^6 motile spermatozoa/mL at 38.5 °C in 5% CO_2 in air with maximum humidity, in Tyrode's salts solution supplemented with 6 mg/mL of BSA, 1.2 mg/mL of HEPES, 1.1 mg/mL of sodium lactate, 0.15 mg/mL of L-glutamine and 0.1 mg/mL sodium pyruvate. After 18 hours, the presumptive zygotes were removed, washed, and

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