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ScienceDirect
Theriogenology 73 (2010) 199–207
Theriogenology
www.theriojournal.com

The effects of roscovitine on cumulus cell apoptosis and the developmental competence of domestic cat oocytes

T. Sananmuang, M. Techakumphu, T. Tharasanit *

Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

Received 7 January 2009; received in revised form 2 August 2009; accepted 30 August 2009

Abstract

The developmental competence of cat oocytes matured in vitro is relatively poor when compared with that of in vivo oocytes. The study aimed to investigate the effect of roscovitine on the developmental competence of cat Felis catus oocytes matured in vitro. Cumulus-oocyte complexes (COCs) were classified as Grade I and II to III. Groups of COCs were cultured in 0, 12.5, 25, 50, 100, and 200 μM roscovitine for 24 h and were either fixed to assess the stages of nuclear maturation (Experiment 1) or additionally matured in vitro for 24 h before fixation (Experiment 2). In Experiment 3, cumulus cells from the COCs treated with roscovitine were examined for apoptosis. Experiment 4 examined the developmental competence of cat oocytes after roscovitine treatment and in vitro fertilization in terms of cleavage and morula and blastocyst formation rates. Roscovitine reversibly arrested cat oocytes at an immature stage in a dose-dependent manner. Roscovitine at 12.5 and 25 μM demonstrated less efficiency compared with that of other doses. However, higher doses of roscovitine induced cumulus cell apoptosis and resulted in a high number of degenerated oocytes after in vitro maturation. Roscovitine at 12.5 and 25 μM were therefore used to evaluate their effect on embryo development. Pretreatment with 12.5 and 25 μM roscovitine prior to in vitro maturation decreased the developmental competence of cat oocytes compared with that of non–roscovitine-treated controls. In conclusion, roscovitine reversibly maintained cat oocytes at the germinal vesicle stage without detrimental effect on nuclear maturation. However, it negatively affected cumulus cell viability and developmental competence.

Keywords: Apoptosis; Cat; Cumulus cells; Embryo; Oocytes; Roscovitine

1. Introduction

The domestic cat is considered to be a valuable model for assisted reproductive biotechnology in nondomestic felids. However, the overall success of in vitro embryo production related to in vitro maturation in this species remains inconsistent. In vivo, mammalian oocytes acquire their nuclear and cytoplasmic maturation during follicle growth [1]. The highly competent dominant follicles are selected and will release oocytes at ovulation, whereas the remaining follicles undergo atresia [2]. In contrast with the in vivo situation, oocytes collected for in vitro maturation originate from follicles at various stages of their growth. In parallel, they demonstrate relatively poor developmental potential compared with that of in vivo–matured oocytes [3]. The quality of the oocyte and cumulus cells is recognized as a potential factor associated with its developmental competence, in terms of the capability of oocytes to resume meiosis, to mature, and to be fertilized and develop up to blastocyst stage in vitro. Cumulus cells surrounding the oocytes also play a critical role in their growth and maturation by providing nutrients and several signals into the oocytes [4–6].

* Corresponding author. Tel.: +66 2 218 9644; fax: +66 2 252 0738.
E-mail address: Theerawat.t@chula.ac.th (T. Tharasanit).

0093-691X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2009.08.013

In domestic cats, only 40% to 60% of immature oocytes reach metaphase II (MII) in vitro, while blastocyst formation rates of 30% to 40% from in vitro–matured oocytes can be achieved [3]. Understanding the mechanism that regulates oocyte maturation thus plays a central role in the optimization of in vitro–maturation technique and in the improvement of embryo production. Until recently, although the exact pathway that regulates the developmental competence of cat oocytes is unclear, the M-phase promoting factor (MPF) and mitogen activated protein (MAP) kinases have been shown to actively induce meiotic progression [7]. Of the many strategies to improve the cytoplasmic maturation of the oocyte, two-step culture using MPF inhibitors, such as butyrolactone and roscovitine, has been developed to increase the developmental competence of immature oocytes from immature follicles. The first step involves the inhibition of the germinal vesicle breakdown, and the second step aims to stimulate the completion of nuclear and cytoplasmic maturation of the oocyte. This technique increased significantly the meiotic competence of cattle oocytes isolated from small antral follicles [8]. Roscovitine, a potent cyclin-dependent inhibitor of the MPF activity that reversibly inhibits meiotic progression, has been examined in many species such as bovine [9,10], goat [11], and pig [12,13]. Although this effect does not compromise the establishment of pregnancy or fetal development during organogenesis [9], in vitro embryo development of roscovitine-treated oocytes has been variable among species studied [10,11,14]. It is therefore hypothesized that roscovitine may affect the in vitro developmental competence of the oocytes in a species-specific manner.

In this regard, prematuration with roscovitine has to be evaluated as a means of improving the developmental competence of cat oocytes, particularly for poor-quality cumulus-oocyte complexes (Grade II to III). This study aimed to examine the effect of roscovitine on cat oocytes, specifically on meiosis inhibition, cumulus cell apoptosis, and developmental competence after in vitro fertilization.

2. Materials and methods

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise indicated.

2.1. Oocyte recovery

Ovaries were collected from domestic cats Felis catus after ovariohysterectomy and transported to the laboratory in NaCl 0.9% in deionized water supplemented with 100 IU/mL penicillin and 100 μg/mL streptomycin. Within 2 h, the ovaries were washed and placed in holding medium (HM) consisting of HEPES-buffered M199, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 4 mg/mL bovine serum albumin (BSA; embryo tested). Cumulus-oocyte complexes were recovered by ovarian mincing in HM and then morphologically classified at × 40 magnification using a stereomicroscope (SMZ645; Nikon, Tokyo, Japan) as described by Wood and Wildt [15]. In brief, Grade I COCs were typified by the oocytes being completely surrounded with more than five layers of compacted cumulus cells and containing a homogeneous-darken ooplasm, while Grade II to III COCs had irregular pale ooplasm surrounded with fewer layers of compacted cumulus cells.

2.2. Oocyte culture

Groups of 20 to 30 COCs were cultured in 500 μL of a basic in vitro maturation medium ($NaHCO_3$-M199 supplemented with 1.0 mM sodium pyruvate, 2.0 mM L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin, 4 mg/mL BSA) containing roscovitine at different concentrations (0, 12.5, 25, 50, 100, and 200 μM) in a 4-well plate (Nunc, Roskilde, Denmark). After this prematuration in roscovitine, in vitro maturation was performed at 38.5 °C in a humidified atmosphere with 5% CO_2 in air for 24 h in an IVM medium (basic in vitro maturation medium supplemented with 0.05 IU/mL recombinant human follicle-stimulating hormone (rhFSH; Organon, Bangkok, Thailand).

2.3. In vitro fertilization

After maturation, the cumulus cells were partially removed by gentle pipetting. In vitro fertilization was performed essentially as described by Pope [16] with minor modifications. In brief, groups of 5 to 10 oocytes were transferred to 50-μL droplets of IVF medium (Tyrode's balanced salt solution containing 1% [vol/vol] Minimum Essential Media (MEM) nonessential amino acids [NEAA], 6 mg/mL BSA, 100 IU/mL penicillin, 30 μg/mL heparin, 1 mM L-glutamine, 0.36 mM sodium pyruvate, and 0.11 mM calcium lactate) [16]. The semen used in this study was collected from two fertility-proven tom cats and then frozen according to Rota et al. [17] with minor modifications. In brief, the cats were anesthetized with 0.04 mg/kg atropine sulfate (A.N.B. Laboratories,