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Short Communication

Is the mouse follicle culture a good model for the goat with respect to the development of preantral follicles in vitro?

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ABSTRACT

The present study evaluated the efficiency of using 2 culture media developed for mice and for goats in the in vitro preantral follicle culture of each species. Murine and caprine secondary follicles were cultured in vitro with human recombinant follicle-stimulating hormone (murine medium) or with bovine recombinant follicle-stimulating hormone in association with growth hormone (caprine medium). The results showed that murine follicles cultured in caprine medium had lower ($P < 0.05$) rates of follicular survival and growth, whereas for caprine follicles, these variables were not affected by the type of medium used ($P > 0.05$). After in vitro maturation, a higher ($P < 0.05$) number of oocytes that resumed meiosis were observed in the murine medium for both species. In contrast, only in the caprine species estradiol production was significantly superior when the caprine medium was used. Higher progesterone production was observed in the presence of the murine medium only for murine follicles ($P < 0.05$). In conclusion, murine and caprine preantral follicles cultured under the same in vitro culture medium conditions respond differently; caprine oocytes grown in vitro in the presence of the murine medium show the greatest developmental competence among the tested combinations. Therefore, under the present experimental conditions, the mouse follicle culture has proved be a good model for the development of new culture media for caprine preantral follicles.

1. Introduction

The murine experimental model is the most efficient for the in vitro manipulation of ovarian follicles [1]. The in vitro maturation and fertilization of preantral follicles can result in the birth of live offspring in mice [2]. Nonetheless, similar results have not been obtained for preantral follicles from humans and livestock, including goats. This situation raises the question of the applicability of experimental models. However, to the best of our knowledge, no comparative study of in vitro follicular development between mice and goats has been performed.

A three-dimensional (3D) in vitro follicle culture system, based on an alginate hydrogel, provided an improvement in the achievement of live offspring in mice, with a 20% rate of live birth [3]. This result was also obtained through the addition of low concentrations (10 mIU/mL) of human recombinant follicle-stimulating hormone (FSH). Conversely, in goats, high concentrations of bovine recombinant FSH, added at increasing concentrations (100 ng/mL until day 6,

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500 ng/mL until day 12, and 1000 ng/mL until day 18 of culture), have been used in the in vitro culture of preantral follicles [4,5], resulting in embryo production. Importantly, the production of the latest caprine embryo stage (morula) reported in the literature from preantral follicles was obtained using medium containing FSH associated with growth hormone (GH) [5]. For a better understanding of the possible interspecies differences and, consequently, of the applicability of the murine follicle culture model to goats, the present study aimed to verify whether mice and goats show similar behaviors during the in vitro culturing of their respective preantral follicles using culture media designed for each species.

2. Materials and methods

Ovaries from prepubertal 12-d-old female Swiss albino mice (*Mus musculus*) (n = 8 ovaries) and adult mixed-breed goats (n = 30 ovaries) were obtained. All experiments were performed according to the recommendations of the Committee of Animal Handling and Ethical Regulation from the State University of Ceara, Fortaleza, Ceara, Brazil. Morphologically normal murine (100–130 μm in diameter; n = 139) and caprine (≥150 μm in diameter; n = 145) secondary follicles were mechanically isolated and encapsulated into sterile 0.5% (wt/vol) alginate (FMC BioPolymers, donated by Dr Teresa K. Woodruff, Evanston, IL, USA) beads as described previously [3] with slight modifications. The basic culture medium consisted of α-MEM (pH 7.2–7.4) supplemented with 3 mg/mL BSA, 1 mg/mL of bovine fetuin, 1% ITS (10 μg/mL insulin, 5.5 μg/mL transferrin, and 5 ng/mL selenium), 2 mM glutamine, 2 mM hypoxanthine, and 50 μg/mL ascorbic acid. For the experimental conditions in both species, secondary follicles were cultured under the following 2 conditions: α-MEM$^+$ plus 10 mIU/mL of human recombinant FSH (National Hormone and Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA; mouse medium [3]) or α-MEM$^+$ plus increasing concentrations of bovine recombinant FSH (Nanocore, SP, Brazil; eg, 100 ng/mL until day 4 [murine follicles] or 6 [caprine follicles], 500 ng/mL until day 8 [murine follicles] or 12 [caprine follicles], and 1,000 ng/mL until day 12 [murine follicles] or 18 [caprine follicles]) in the presence of 50 ng/mL of GH (MP Biomedicals, Solon, OH, USA; caprine medium [5]). The in vitro cultures were replicated 2 times for mice and 3 times for goats, and a total of at least 69 (murine) or 70 (caprine) follicles were used for each treatment condition. Alginate beads containing a single follicle were plated at one follicle per well in 96-well plates in 100 μL of culture medium for the murine follicles or in 48-well plates in 300 μL of culture medium for the caprine follicles. The encapsulated murine and caprine follicles were cultured at 37°C for 12 d or at 39°C for 18 d, respectively and in 5% CO_2 for both species. Every other day, half of the medium (50 μL or 150 μL) was exchanged. Follicles showing darkness of the oocytes and surrounding cumulus cells or those with misshapen oocytes were classified as degenerated. The follicular diameter was measured only in healthy follicles every 4 (murine follicles) or 6 (caprine follicles) days of culture. Antral cavity formation was defined as a visible translucent cavity within the granulosa cell layers. The daily increase in follicular diameter was calculated as follows: the diameter of viable follicles at day 12 (murine) or 18 (caprine) minus the diameter of viable follicles at day 0 divided by the number of days of in vitro culture (12 or 18 d). At the end of the culture period, the culture medium was replaced by α-MEM containing 10-mIU/mL alginate lyase for 30 min at 37°C or 39°C. All healthy murine and caprine follicles containing oocytes with homogeneous cytoplasm surrounded by at least 1 compact layer of cumulus cells were selected for in vitro maturation. Mouse and goat cumulus oocyte complexes were transferred to maturation medium as described previously [3,5], where they were cultured for 16 h at 37°C or for 35 h at 39°C, respectively, and in 5% CO_2 for both species. Subsequently, oocytes were analyzed by fluorescence microscopy to assess the viability and chromatin configuration using calcein-AM, ethidium homodimer-1, and Hoechst 33,342 as markers. The spent medium was collected every 4 or 6 d for murine and caprine follicles, respectively, during a culture period of 12 or 18 d and stored at −20°C for subsequent estradiol and progesterone assays using double antibody radioimmunoassay (MP Biomedicals). Data for discrete variables (follicular survival, antrum formation, and meiotic resumption) were analyzed as the dispersion of frequency by Chi-squared test. The results for continuous variables (follicle diameters and steroid levels) did not show homoscedasticity among treatments (even after transformation of data) and were analyzed by the Kruskal–Wallis nonparametric test. The results were expressed

Table 1
Follicular survival (%), antrum formation (%), and meiotic stages of oocytes (%) from murine and caprine ovarian follicles cultured for 12 or 18 d, respectively, in murine or caprine media.

Source of preantral follicles	Mouse		Goat	
Type of medium	Murine medium	Caprine medium	Murine medium	Caprine medium
N° follicles	69	70	75	70
Survival (%)	95.65[a]	72.86[b]	60.00[a]	57.14[a]
Antrum formation (%)	55.07	0.00	60.00[a]	51.43[a]
Selected for IVM (%)	48/69 (69.57)[a]	40/70 (57.14)[a]	37/75 (49.33)[a]	33/70 (47.14)[a]
GVBD N° (%)	34/48 (70.83)[a]	18/40 (45.00)[b]	12/37 (32.43)[a]	3/33 (09.09)[b]
MII N° (%)	20/48 (41.67)[a]	6/40 (15.00)[b]	1/37 (2.70)	0.00

Abbreviations: GVBD, germinal vesicle breakdown; IVM, in vitro maturation; MII, oocytes in metaphase II.
[a,b] Within a row, groups without a common superscript differed ($P < 0.05$) between murine and caprine media within the same species.