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Effect of caprine ovarian follicular fluid on maturation of caprine oocytes *in vitro*

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ABSTRACT

The study was conducted to evaluate the effect of caprine follicular fluid on the maturation of caprine oocytes in vitro. The follicular fluid was collected by aspiration of visible follicles of size >3mm in diameter from slaughter house ovaries. The caprine ovaries were collected immediately after slaughter and cumulus oocyte complexes were retrieved by aspiration followed by slicing , graded and subjected to in vitro maturation in 25, 50 and 100 per cent concentration of caprine follicular fluid. The basic culture medium (Control) comprised of Tissue culture medium (TCM199), BSA (3mg/ml) and Gentamicin (50µg/ml). Only grade A and B were placed in the culture medium in Petri dishes with overlaying mineral oil and incubated for 24 h at 38ºC, 5%CO_2 in 95 per cent relative humidity. The maturation was evaluated by cumulus expansion score and extrusion of first polar body.

The maturation rate of caprine oocytes in 25,50, and 100 per cent concentration of caprine follicular fluid were 78.50±2.4,77.63± 2.57 and 83.65±3.59 respectively which was significantly higher than those that obtained in control (55.08±3.76). It can be concluded that addition of follicular fluid to culture medium enhances the maturation of caprine oocytes in vitro.

Keywords: Follicular fluid, caprine, in vitro, maturation, polar body

I. INTRODUCTION

Since the cost of production of embryos is very high, several efforts are being made to reduce the cost of technology by replacing the conventionally used fetal bovine serum and hormones with steer serum, cow serum or follicular fluid (Gupta *et al*., 2001).The beneficial effect of inclusion of follicular fluid in medium for *in vitro* maturation of oocytes and their subsequent fertilization and development has been reported in pig (Naito, 1988), sheep (Sun *et al* ., 1994) and cows (Kim *et al*., 1996) embryos. The information on utilization of follicular fluid of caprine on *in vitro* maturation of caprine oocytes is lacking and hence this study was carried out to evaluate the effect of different concentration of caprine follicular fluid on *in vitro* maturation of caprine oocytes

II. Materials and Methods

The goat ovaries required for the study was obtained from Karnataka Meat and Poultry Marketing Corporation slaughter house, Bangalore and were transported to the laboratory within one hour in a thermos flask containing warm (28-30ºC) sterile physiological saline supplemented with 50µg/ml gentamicin sulphate.

A. Collection and processing of follicular fluid: Follicular fluid was aspirated from follicles having diameter >3mm using sterile 18G needle fitted to 5ml disposable syringe. The required quantity of follicular fluid was pooled and centrifuged at 5000 rpm for 30 minutes. The supernatant was collected and heat inactivated at 56ºC for 30 min, cooled to room temperature and filtered through 0.2µm filter and stored in 2ml micro centrifuge tube at -20ºC until use.

B. Collection and grading of oocytes: The oocytes were retrieved by aspiration followed by slicing technique. The oocytes were graded as described by Singh and Sharma (1991) as grade A: having more than three layers of cumulus cells, grade B: those with 2-3 layers of cumulus cells, grade C: those with one layer of cumulus cells or having scattered envelope of cumulus cells, grade D: without any cumulus cells. Only Grade A and B oocytes were cultured *in vitro* for 24 h at 38ºC, 5% CO_2 and 95 per cent relative humidity. Grade A and B were considered as culturable and Grade C and D as non-culturable. The maturation was assessed by cumulus expansion score and visualization of first polar body. Degree of cumulus expansion was graded according to Downs (1989), Harper and Benjamin (1993) and Gupta *et al*., (2005) as 0: no expansion, 1: cumulus expansion involving only outermost layer, 2: partial cumulus expansion involving all the layers except corona radiate, 3: cumulus expansion involving all the layers including corona radiata. A random sample of oocytes with grade 2 and 3 cumulus expansion were denuded and observed for first polar body under invert microscope (Nikon,DIAPOT–TMD,Japan). A first polar body was found in all oocytes with grade 2 and 3 cumulus expansion . Hence all the grade 2 and 3 oocytes were considered matured (Gupta *et al.*, 2005). In addition, all the grade 0 and 1 cumulus expansion oocytes were also observed for extrusion of first polar body. If a polar body was detected such oocytes were also considered as matured for the purpose of calculation of total maturation rate of oocytes (Nandi *et al.*, 2003).

Statistical analysis: Maturation rates of different grades was analyzed by ANOVA followed by Tukey's test. The total maturation rate was calculated by ANOVA followed by Tukey's multiple comparison test. The statistical package of graph pad prism, San Diego,USA was considered for analyzing the data. Difference between the mean values was significant when P values were less than 0.05.

III. RESULTS AND DISCUSSION

The recovery rate of different grades of oocytes namely A, B, C and D by follicular aspiration followed by slicing were 1.05±0.07, 0.62±0.04, 0.56±0.05, and 0.46±0.05 respectively. The results revealed significantly higher ($P \leq 0.05$) rate of grade A oocytes than other three grades. No difference was observed between grade B, C and D oocytes recovered. It was observed that a significant difference existed between culturable (1.67±0.10) and non culturable oocytes (1.03±0.09) per ovary. The overall recovery rate of oocytes obtained in the study (2.68±0.15) is more or less similar to that obtained by Pawshe *et al.,(*1994) and Kothandaraman and Veerapandian (2005) which was 2.40 and 2.88 respectively by slicing method. However, Naqui *et al* ., (1992) recorded 4.08 per ovary might be due to personnel experience, dexterity of the person, breed of animal, stage of estrous cycle, season, nutritional status and laboratory conditions. The maturation rates of caprine oocytes in different concentration of follicular fluid are presented in table 1.The maturation rates were compared with control. The maturation rate in control are in agreement with that of Agarwal (1992) and Pawshe *et al* . (1993) who recorded a maturation rate of 54.79 and 53.00 per cent respectively. Further, it was observed that there was no significant difference between 25, 50 and 100 per cent concentration of caprine follicular fluid on total maturation of caprine oocyte. However, significantly higher total maturation was observed with all the three different levels of caprine follicular fluid when compared with that recorded in control. There is no available literature on the effect of follicular fluid on maturation of caprine oocytes for comparative discussion. In the present study it was observed that there was tendency for higher maturation percentage in 100 per cent caprine follicular fluid.

Table 1. Maturation of caprine oocytes with different concentration of caprine follicular fluid (cFF)

Conc. of cFF	No. of oocytes cultured	Maturation grading on Cumulus Expansion(Mean±SE)				Polarbody in grade0 & grade1matured oocytes (Mean%±SE)	Total maturation (Mean%±SE)
		0	1	2	3		
Control (TCM199)	201	96(46.75±4.60)	23(12.48±2.4)	28(12.43±1.34)[a]	54(28.40±4.44)[b]	38(25.87±7.85)	120(55.09±3.76)[b]
25%	212	41(20.42±1.99)	23(12.22±1.96)	42(18.30±3.05)[a]	106(49.07±3.30)[a]	23(37.26±3.75)	171(78.50±2.4)[a]
50%	212	50(22.98±4.53)	30(14.26±2.23)	40(18.13±2.98)[a]	92(44.61±4.74)[ba]	34(40.98±4.53)	166(77.63±2.57)[a]
100%	214	46(22.51±8.06)	20(7.45±3.35)	45(22.08±3.67)[a]	103(47.98±5.36)[ca]	32(38.74±10.52)	180(83.66±3.59)[a]

Values in the parenthesis indicate percentage
Means bearing any one common superscript within in the column did not vary significantly (P≤0.05)
Total maturation rate includes grade 2 and 3 cumulus expanded Cumulus Oophorus Complex (COC) plus Grade 0 and 1 cumulus expanded COC in which first Polar body (PB) was observed.

***Figure 1*.Grade 3 Cumulus expansion**

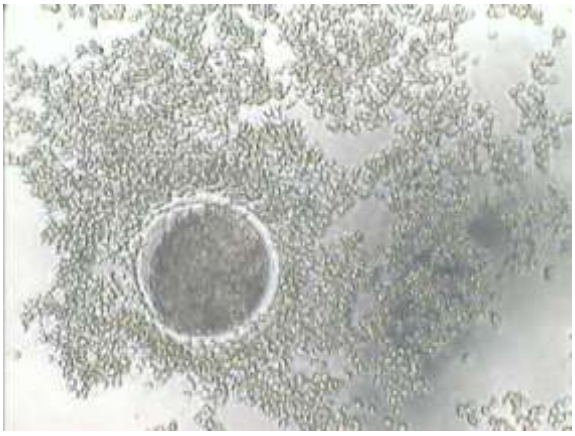

***Figure 2*. Extrusion of polar body**

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