EFFECT OF RETRIEVAL TECHNIQUE AND CUMULUS OOCYTE MORPHOLOGY ON *IN VITRO* MATURATION OF CAPRINE FOLLICULAR OOCYTES*

Ambili John[1], Metilda Joseph[2] and V. Vijayakumaran[3]
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy-680 651, Thrissur, Kerala.

Received:15.06.2014
Accepted:01.08.2014

bstract

A study was carried out on in vitro maturation of caprine follicular oocytes using oocytes obtained by three retrieval techniques namely aspiration, slicing and puncture; from ovaries of Malabari goats and its crossbreds collected immediately after slaughter. A total of 138 ovaries were processed and oocytes obtained were classified into different grades based on number of cumulus cell layers and ooplasm character as A, B, C and poor quality. A, B and C class oocytes obtained by different retrieval methods were cultured separately in 50 µl drops of maturation media in a standard water-jacketed type of CO_2 incubator with a temperature of 38.5º C, five per cent CO_2 tension and maximum humidity. After 24 h of culture, oocytes were examined for maturational changes. Retrieval method was found to have no significant effect, whereas, the cumulus oocyte morphology had significant effect on in vitro maturation rate.

Key words: *Goat, oocyte retrieval, cumulus expansion, maturation*

Vast and diverse genetic resources of goats are found in India and among this, Malabari breed, a native of Kerala, is well known for its milk production and adaptability. This small ruminant plays a major role in the rural economy of the state. Recently, there is an accelerated interest for goat rearing in Kerala as evidenced by the increase in goat population as against a decline in cattle and buffalo (AHD, 2007). But in the present scenario of increased fragmentation of land holdings, diversion of land for non-agricultural purposes and increased interest of farming community in other profitable enterprises, it has become inevitable to make goat rearing more profitable. Hence, adoption of advanced reproductive technologies to enhance profitability of goat rearing is the need of the hour.

Economical means for *in vitro* embryo production can be expected to play a prominent role in future goat breeding strategies (Ishwar and Menon, 1996). *In vitro* production (IVP) of embryos is currently the central focus in livestock industry including goats. For the successful IVP

**Part of MVSc thesis of first author submitted to Kerala Agricultural University, Thrissur 680 656, Kerala, India*
1. *Ph.D Scholar*
2. *Associate Professor*
3. *Professor (Retd.)*

programme in goat and other livestock, *in vitro* maturation (IVM) of cumulus oocyte complex (COC) is a primary requirement.

A study was carried out on *in vitro* maturation of caprine follicular oocytes using oocytes obtained by three retrieval techniques from ovaries of recently slaughtered animals.

Materials and Methods

Ovaries dissected out from freshly slaughtered goats belonging to Malabari breed and its crosses were transported to the laboratory within 1-2 h in a thermo flask containing freshly prepared normal saline solution fortified with antibiotics; 100 IU/ml benzyl penicillin and 100 µg/ml streptomycin sulphate (Chauhan and Anand, 1991). A total of 138 ovaries were processed during the study. After repeated washing of ovaries in normal saline and final washing in Dulbeco's Phosphate Buffered Saline (DPBS), oocytes were recovered by aspiration, slicing or puncture into cumulus oocyte complex (COC) handling medium (DPBS enriched with five per cent day zero oestrus goat serum and 0.5 per cent BSA) maintained at 38ºC (Binoy, 2009). A total of 550 oocytes were recovered by the three retrieval techniques.

Recovered oocytes were rinsed several times in COC handling medium. These oocytes were classified into different quality grades based on number of cumulus cell layers and ooplasm character as A, B, C and poor quality grades (Fig 1). Grade A COCs were morphologically normal with more than three complete layers of compact cumulus cells and uniform granulation of ooplasm. Grade B oocytes had normal morphology with two to three complete layers of cumulus cells and uniform granulation of ooplasm. Oocytes in the C grade were morphologically normal with single complete layer of cumulus cells and uniform granulation of ooplasm. All other oocytes that were found to be inferior to grade C quality were graded as poor quality oocytes (Vijayakumaran, 1995).

A, B and C class oocytes obtained by different retrieval methods were cultured separately in 50 µl drops of maturation media. Medium used for *in vitro* maturation of oocytes was freshly prepared Hepes modified TCM- 199 (25 mM Hepes) enriched with 22 µg /ml sodium pyruate, 2.2 mg /ml sodium bicarbonate and penicillin G sodium and streptomycin sulphate at the rate of 500 µg /ml each. This medium was further supplemented with hormones, 1 µg /ml estradiol- 17β, 0.5 µg /ml FSH, 0.06 IU hCG and 20 per cent heat- inactivated oestrus goat serum prior to employing it for IVM (Kharche *et. al.,* 2006). Culture conditions were set in a standard water-jacketed type of CO_2 incubator with a temperature of 38.5º C, five per cent CO_2 tension and maximum humidity. After 24 h of culture, oocytes were examined for maturational changes like cumulus expansion. A total of 90 oocytes, 30 each from each retrieval method showing cumulus expansion were stained with one per cent aceto-orcein and examined under bright field of inverted phase contrast microscope at 400x magnification and assessed the level of meiotic progression.

The data obtained were subjected to statistical analysis as per Snedecor and Cochran (1996).

Results and Discussion

The details of cumulus expansion and nuclear maturation rates of different grades of oocytes collected by aspiration, slicing and puncture are presented in Tables 1 and 2.

Values with different superscripts (a,b) in the same row differ significantly (P<0.01)

Out of 431 oocytes subjected to *in vitro* maturation, 248 exhibited cumulus expansion (Fig. 2) and out of 90 oocytes examined for nuclear maturation, 23 exhibited metaphase II (MII) stage (Fig. 3).

No significant difference was observed between aspiration and puncture in the cumulus expansion rates of different classes of oocytes. B class oocytes retrieved by slicing exhibited significantly lower cumulus expansion rate than B class oocytes obtained by aspiration or puncture (Table 1). With regard to quality of oocytes, A grade oocytes exhibited significantly higher cumulus expansion rates than B or C class oocytes (Table 3).

In the present study, it was observed that retrieval method had no significant effect on cumulus expansion potential of caprine oocytes, whereas, the COC morphology had significant effect on the same. Similarly, Vijayakumaran (1994) reported that retrieval technique had no significant effect whereas oocyte quality significantly affected the maturation rate of

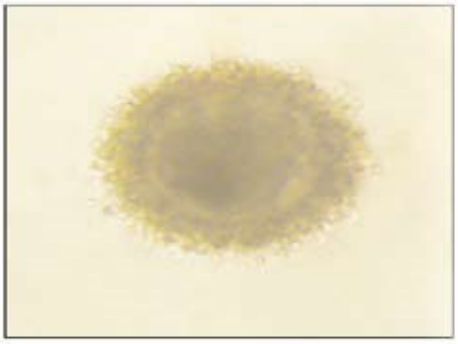
Table 1. Effect of oocyte retrieval technique on cumulus expansion rate of oocytes

Grade of COCs	Oocyte retrieval systems								
	Aspiration			Slicing			Puncture		
	Oocytes kept for maturation	Oocytes showing cumulus expansion	Expanded COCs (%)	Oocytes kept for maturation	Oocytes showing cumulus expansion	Expanded COCs (%)	Oocytes kept for maturation	Oocytes showing cumulus expansion	Expanded COCs (%)
A	44	35	77.75[a]	50	35	69.70[a]	50	36	71.49[a]
B	44	28	63.48[a]	60	31	51.37[b]	52	33	63.39[a]
C	41	16	39.17[a]	52	20	32.57[a]	38	14	37.29[a]

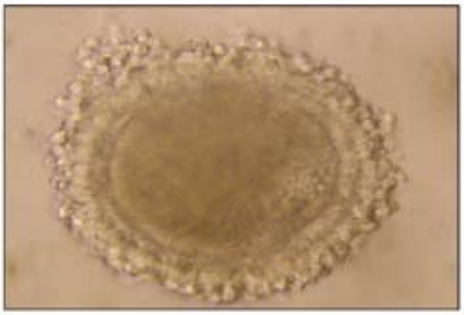
Values with different superscripts (a,b) in the same row differ significantly (P<0.05)

Fig.1. Classifcation of cumulus-oocyte complexes

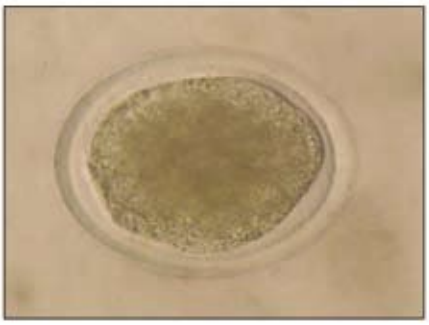
A grade Oocyte (200x)



B grade Oocyte (200x)



C grade Oocyte (200x)



Poor Quality Oocyte (200x)

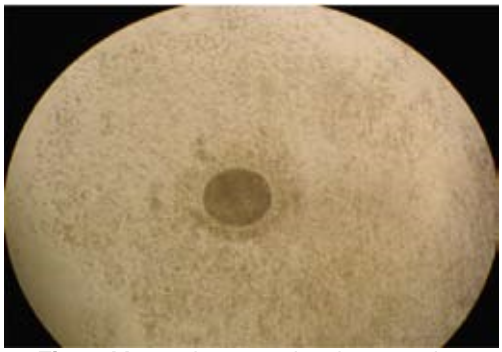


Fig. 2. Matured oocyte showing cumulus expansion (200x)

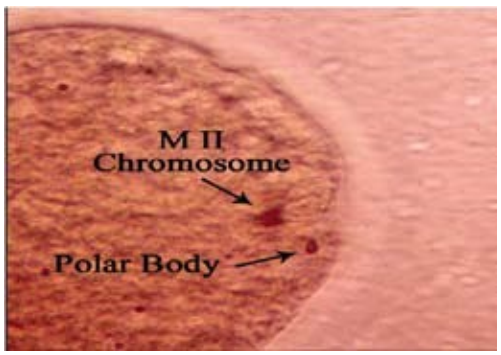


Fig. 3. Oocyte showing metaphase II plates and polar body

Table 2. Effect of oocyte retrieval technique on nuclear maturation rate of oocytes

Grade of COCs	Oocyte retrieval systems								
	Aspiration			Slicing			Puncture		
	No. of ooctyes examined	No. of MII stage oocytes	Percentage of nuclear maturation	No. of ooctyes examined	No. of MII stage oocytes	Percentage of nuclear maturation	No. of ooctyes examined	No. of MII stage oocytes	Percentage of nuclear maturation
A	10	4	40^{ab}	10	3	30^{b}	10	5	50^{a}
B	10	2	20^{a}	10	2	20^{a}	10	3	30^{a}
C	10	1	10^{a}	10	1	10^{a}	10	2	20^{a}

Table 3. Effect of cumulus oocyte morphology on cumulus expansion and nuclear maturation rates of oocytes

Grade of COCs	Cumulus expansion			Nuclear maturation		
	Oocytes kept for maturation	Oocytes showing cumulus expansion	Cumulus expansion rate (%)	No. of ooctyes examined	No. of MII stage oocytes	Nuclear maturation rate (%)
A	144	106	73.61^{a}	30	12	40.0^{a}
B	156	92	58.97^{b}	30	7	23.33^{b}
C	131	50	38.16^{c}	30	4	13.33^{c}

Values with different superscripts (a,b,c) in the same column differ significantly (P<0.05)

caprine oocytes. Martino *et al.* (1994) observed lower maturation rate for goat oocytes collected by the slicing method and better cumulus expansion rate was reported for oocytes with three or more layers of cumulus cells than those with less than three layers of cumulus cells.

Regarding the nuclear maturation rates obtained in this study, no significant difference was observed between aspiration, slicing and puncture for B and C classes of oocytes. A-class oocytes collected by aspiration and slicing exhibited significantly lower nuclear maturation rate than puncture (Table 2). Considering the quality of oocytes, A grade oocytes exhibited significantly higher ($p<0.05$) nuclear maturation rates than B or C-class oocytes (Table 3).

Analysis of results revealed that the method of retrieval is having no effect on nuclear maturation rate of caprine oocytes. Pawshe *et al.* (1994) observed no significant difference in the number of oocytes maturing to metaphase II at 24 hour culture of oocytes obtained by aspiration, slicing or puncture. Wani *et al.* (2000) reported similar results in sheep. Wang *et al.* (2007) reported significantly lower rate of oocytes reaching the metaphase II stage when using slicing techniques, compared to the other techniques in goats. But the COC morphology is having significant effect on nuclear maturation and polar body extrusion rates. Oocytes with three or more layers of cumulus cells exhibited better nuclear maturation and polar body extrusion rate.

It was concluded from the present study that, retrieval method has no significant effect on cumulus expansion and nuclear maturation rates of different grades of oocytes, whereas cumulus oocyte morphology significantly affects both cumulus expansion and nuclear maturation rates.

References

AHD (Animal Husbandry Department). 2007. Kerala XVIII Quinquennial Livestock Census-2007. Statistical unit, Animal Husbandry Department, Thiruvananthapuram. pp. 1-2.

Binoy, V.S. 2009. *In vitro* fertilization of bovine oocytes using fresh, frozen and epididymal spermatozoa. M.V.Sc. thesis. Kerala Agricultural University, Thrissur, p.122.

Chauhan, M.S. and Anand, S.R. 1991. *In vitro* maturation and fertilization of goat oocytes. *Indian J. Expt. Biol.* **29**: 105-110.

Ishwar, A.K. and Menon, M.A. 1996. Embryo transfer in sheep and goats: A review. *Small Rum. Res.* **19**: 35-43.

Kharche, S.D., Goel, A.K., Jindal, S.K. and Sinha, N.K. 2006. *In vitro* maturation

of caprine oocytes in different concentrations of estrous goat serum. *Small Rum. Res*. **64**: 186-189.

Martino, A., Palomo, M.J., Mogas, T. and Paramio, M.T. 1994. Influence of the collection technique of prepubertal goat oocytes on *in vitro* maturation and fertilization. *Theriogenology*. **42**: 859-873.

Pawshe, C.H., Totey, S.M. and Jain, S.K. 1994. A comparison of three methods of recovery of goat oocytes for *in vitro* maturation and fertilization. *Theriogenology*. **42**: 117-125.

Snedecor, G.W. and Cochran, W.G. 1996. *Statistical Methods.* (8th Ed.). Iowa State University Press, USA. p. 534.

Vijayakumaran, V. 1995. Effect of follicular size on *in vitro* maturation of goat oocytes. PhD thesis, Tamil Nadu Veterinary and Animal Sciences University, Madras, p.185.

Wang, Z.G., Xu, Z.R. and Yu, S.D. 2007. Effects of oocyte collection techniques and maturation media on *in vitro* maturation and subsequent embryo development in Boer goat. *Czech. Anim. Sci.* **52**: 21-25.

Wani, N.A., Wani, G.M., Khan, M.Z. and Salahudin, S. 2000. Effect of oocyte harvesting techniques on *in vitro* maturation and *in vitro* fertilization in sheep. *Small Rum. Res*. **36**: 63-67.

■