

Effect of Oocyte Retrieval Techniques on Yield and Quality of Caprine Oocytes

Ambili John¹, Metilda Joseph², V. Vijayakumaran³ and C. J. Manoj⁴

Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Kerala, 680 651

Abstract: *The efficiency of three oocyte collection methods from goat ovaries was assessed. Oocytes were collected by aspiration (n=165), slicing (n=213) and puncture (n=172). The mean yield of oocytes per ovary was found to be 3.93 ± 0.11 , 4.44 ± 0.06 and 3.59 ± 0.07 by aspiration, slicing and puncture respectively. Slicing yielded significantly higher number of oocytes per ovary. Percentage yield of culturable quality oocytes (A, B and C) by different retrieval methods were 78.18, 76.05 and 81.39 respectively by aspiration, slicing and puncture. It was found that puncture technique yielded significantly higher culturable quality oocytes than aspiration and slicing.*

Keywords: *goat, oocyte, aspiration, slicing, puncture*

I. Introduction

Oocytes are the main raw materials for in vitro embryo production (IVP) experiments. Therefore the success of any IVP programme in goat production largely depends on the continuous supply of quality oocytes in optimum quantity. A number of methods are currently used for oocyte recovery from live or slaughtered goats. In vivo matured oocytes are obtained either by surgical or laparoscopic methods. These methods are expensive and the number of oocytes recovered per ovary is very small (1). Ovaries from slaughtered animals are the cheapest and the most abundant source of primary oocytes. Four methods for collection of oocytes have been described in domestic animals viz. dissection of ovarian follicles, aspiration of the oocyte from the follicle, slicing the ovaries and puncture of visible surface follicles (2). The objective of the study was to identify the most efficient method of oocyte recovery among aspiration, slicing and puncture from goat ovaries.

II. Materials And Methods

Ovaries required for the study were collected from the corporation slaughter house, Kuriachira, Thrissur and transported to the laboratory within 1-2 h of slaughter in a thermo flask containing freshly prepared normal saline solution fortified with 100 IU/ml Benzyl penicillin and 100 µg/ml Streptomycin sulphate at 35-37°C (3). The ovaries were washed in normal saline solution to remove excess blood and tissue debris. After trimming of the extraneous tissue, the ovaries were washed several times in sterile normal saline solution containing penicillin and streptomycin at 37°C. The final washing was done with Dulbecco's Phosphate Buffered Saline (DPBS) supplemented with 0.5 per cent Bovine Serum Albumin (BSA) and maintained at 36-38°C (4).

Oocytes were retrieved from the ovaries by applying any one of the three retrieval methods namely aspiration, slicing and puncture, in Cumulus Oocyte Complex (COC) handling media prepared with DPBS enriched with five per cent day zero oestrus goat serum and 0.5 per cent BSA maintained at 38°C. Out of 138 ovaries collected, 42, 48 and 48 were processed using retrieval methods namely aspiration, slicing and puncture respectively. The medium containing oocytes from all retrieval methods were transferred into separate sterile 90 mm petri dishes having grid and examined under zoom stereomicroscope at 10x magnification for identification of oocytes. Identified oocytes were collected by means of unopette and transferred into 35 mm petri dish containing fresh COC handling media maintained at 38°C. The total yield of oocytes from different retrieval systems and their morphological grades were recorded separately.

All the oocytes obtained by different retrieval systems were examined separately under 40x magnification of zoom stereomicroscope for their morphological character. Based on the number of layers of cumulus cells and ooplasm character (5), the oocytes were graded into three categories viz. grade A grade B and grade C. All other oocytes that were found to be inferior to grade C quality were considered as poor quality.

III. Result

A total of 138 ovaries were subjected to the study which on harvest by three retrieval methods viz. aspiration, slicing and puncture yielded a total of 550 oocytes. Forty two ovaries were subjected to aspiration and 48 each were subjected to slicing and puncture. The yield of oocytes under each retrieval method was 165 by aspiration, 213 by slicing and 172 by puncture (Table 1). Average yield of COC per ovary by aspiration,

slicing and puncture was 3.93 ± 0.11 , 4.44 ± 0.06 and 3.59 ± 0.07 respectively (Table 1). Slicing yielded significantly more oocytes per ovary than aspiration or puncture

Yield of different quality grades of oocytes retrieved by aspiration, slicing and puncture is given in Table 2. Percentage yield of A grade oocyte by aspiration, slicing and puncture was 26.74, 23.08 and 29.01 respectively. The corresponding values for B, C and poor quality oocytes were 26.62, 28.15, 30.26; 24.77, 24.44, 22.07 and 21.87, 23.96, 18.66 respectively. Mean yield of oocytes per ovary by aspiration, slicing and puncture were 1.05 ± 0.05 , 1.04 ± 0.04 , 1.04 ± 0.04 ; 1.05 ± 0.08 , 1.25 ± 0.05 , 1.08 ± 0.03 ; 0.98 ± 0.07 , 1.08 ± 0.07 , 0.79 ± 0.04 and 0.86 ± 0.04 , 1.06 ± 0.06 , 0.67 ± 0.03 respectively for A, B, C and poor quality grades. Percentage yield of culturable quality oocytes (A, B & C) by different retrieval methods were 78.18, 76.05 and 81.39 respectively by aspiration, slicing and puncture. It was found that puncture technique yielded significantly higher culturable quality oocytes than aspiration and slicing.

IV. Discussion

In the present experiment significantly higher COC per ovary was obtained by slicing method, when compared to aspiration or puncture. This is in agreement with the observations of Martino et al. (1994), Vijayakumaran (1995) and Wang et al. (2007) in goats. The reason for more COC yield per ovary in slicing could be attributed to the fact that by slicing, oocytes from surface follicles as well as follicles of deeper cortical stroma are released, whereas by puncture and aspiration oocytes from surface follicles alone are released (Das et al., 1996 and Pawshe et al., 1994). So this experiment points to the fact that as far as the oocyte recovery rate is concerned maximum efficiency is for slicing method than aspiration or puncture.

Yield of culturable quality oocyte was highest with puncture followed by aspiration and slicing. This finding is in agreement with the results of Vijayakumaran (1995), Kharche et al. (2006) and Wang et al. (2007). Pawshe et al. (1994) obtained a comparatively lower percentage of culturable quality oocytes by puncture than the present study. Lower yield of good quality oocytes by slicing method may be due to the damage caused by the blade used for the technique.

Many factors have been found to affect the yield and quality of oocytes viz. breed, season, time interval from collection of ovaries to oocyte harvest, temperature of media for transport of ovaries, retrieval technique and the criteria used for classification of culture grade oocytes (9). Nutritional status, stage of estrous cycle and agro-climatic conditions in which goats are reared also influence oocyte yield and quality. This study revealed that among retrieval techniques, slicing yielded maximum number of oocytes per ovary, while per cent yield of culturable quality oocytes was significantly higher by puncture method.

Acknowledgement

The authors extend their gratitude to Dr. Joseph Mathew (Associate Professor –Retd) and Dr. Hiron M Harshan (Assistant Professor), Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy for their technical support during the study. We would also like to thank the Dean, College of Veterinary and Animal Sciences and Kerala Agricultural University for providing the fund necessary for conducting the work.

References

- [1]. 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
- [2]. Das, G. K., Jain, G. C., Solanki, V. S. and Tripathi, V. N. 1996. Efficacy of various collection methods for oocyte retrieval in buffalo. *Theriogenology*. 46: 1403-1411
- [3]. Chauhan, M. S. and Anand, S. R. 1991. In vitro maturation and fertilization of goat oocytes. *Indian J. Expt. Biol.* 29:105-110
- [4]. 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
- [5]. Vijayakumaran, V. 1995. Effect of follicular size on in vitro maturation of goat oocytes. Ph.D thesis, Tamil Nadu Veterinary and Animal Sciences University, Madras, p. 185
- [6]. 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
- [7]. 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
- [8]. 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
- [9]. Rahman, A. N. M. A., Abdullah, R. B. and Wan-Khadijah, W. E. 2008. Recovery and grading of goat oocytes with special reference to laparoscopic ovum pick-up technique: A review. *Biotechnology*. 7:612-622

Table 1. Effect of three harvesting techniques on total oocyte yield and Mean (\pm S.E) recovery rate of oocytes per ovary

Sl. No.	Retrieval methods	Number of ovaries	Total yield of oocytes	Mean number of oocytes (Mean \pm S.E)
1	Aspiration	42	165	3.93 \pm 0.11 ^a
2	Slicing	48	213	4.44 \pm 0.06 ^b
3	Puncture	48	172	3.59 \pm 0.07 ^c
4	Total	138	550	3.99 \pm 0.09 ^d

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

*Indicate average

Table 2. Effect of retrieval techniques on yield of different quality grades of oocytes

Sl. No.	Morphological grade of COCs	Oocyte retrieval systems					
		Aspiration		Slicing		Puncture	
		Percentage of total yield	Mean number per ovary	Percentage of total yield	Mean number per ovary	Percentage of total yield	Mean number per ovary
1	A	26.74 ^a	1.05 \pm 0.05	23.08 ^b	1.04 \pm 0.04	29.01 ^a	1.04 \pm 0.04
2	B	26.62 ^a	1.05 \pm 0.08	28.15 ^{ab}	1.25 \pm 0.05	30.26 ^b	1.08 \pm 0.03
3	C	24.77 ^a	0.98 \pm 0.07	24.44 ^a	1.08 \pm 0.07	22.07 ^a	0.79 \pm 0.04
4	Poor	21.87 ^{ab}	0.86 \pm 0.04	23.96 ^b	1.06 \pm 0.06	18.66 ^a	0.67 \pm 0.03
5	Culturable Quality (A, B & C)	78.18 ^a	3.07 \pm 0.10	76.05 ^a	3.37 \pm 0.05	81.39 ^b	2.92 \pm 0.06

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