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Research Article

Assessment of sheep oocyte quality for *in vitro* maturation*

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ABSTRACT

Sheep ovaries were collected from slaughter house and transported to the laboratory within 30 min. A total of 2117 oocytes were retrieved using slicing technique with a recovery rate of 4.91 oocytes per ovary. Among the collected oocytes 33.87 (717), 32.73 (693) and 33.40 (707) per cent of oocytes were graded as A, B and C, respectively. The mean maturation percentage of A, B and C grade oocytes in the present study was 95.48 ± 0.63, 89.14 ± 1.17 and 77.94 ± 1.12, respectively. The maturation rate of A grade oocytes were significantly (<0.01) higher than that of B and C grades and B grade oocytes were significantly (<0.01) higher than that of C grade oocytes. The overall maturation rate of oocytes in the present study was 87.5 ± 1.08 per cent.

Key words: Sheep, Oocytes, *In vitro* maturation

INTRODUCTION

In vitro embryo production provides an excellent source of low-cost embryos for basic research on developmental biology and for commercial application of the emerging biotechnologies. The quantity and quality of oocytes retrieved per ovary is an important consideration in the production of *in vitro* embryos. The most commonly used oocyte recovery method in sheep is slicing (Wahid *et. al.*, 1992), aspiration of visible follicles and follicular dissection. The present study was conducted to examine the effect of slicing on the quantity, quality and maturation of oocytes.

MATERIALS AND METHODS

Retrieval of oocytes

Ovaries were collected from ewes of mixed breeds, irrespective of age, body condition, stage of estrous cycle and season from a slaughter house and transported to the laboratory within 30 min, in warm (37°C) phosphate buffered saline (PBS) supplemented with 50 ìg/ml gentamicin sulphate. The extra ovarian tissues were trimmed and washed three times with tap water and five times in PBS. The ovaries were sliced as per the standard technique described by Datta *et al* (1993) in a 60 mm petridish containing oocyte collection medium (modified HEPES- buffered Tyrodes medium). The cumulus oocyte complexes (COC) were isolated under a stereo zoom microscope and then graded as A, B, C according to Wahid *et. al.* (1992).

In vitro maturation (IVM) of oocytes

The IVM of oocytes was done using TCM-199 supplemented with 10 per cent fetal bovine serum (FBS), 200 ìM cysteamine, 1.0 ìg/ml follicle stimulating hormone (FSH), 0.02 ìg/ml luteinizing hormone (LH), 1 ìg/ml 17-â estradiol and 50 µg/ml gentamicin. The graded oocytes rinsed four times in maturation medium and transferred to 2 h pre equilibrated 50 ìl of IVM droplets (10 COCs / droplet) overlaid with sterile mineral oil. The

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oocytes were allowed to mature in these droplets at 38.5°C in an atmosphere of 5 per cent CO_2 for 24 h in a CO_2 incubator. The maturation was assessed based on the expansion of the cumulus cells (Kobayashi *et. al.*, 1994) and extrusion of the first polar body under the stereo zoom microscope. Data were analysed by ANOVA (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Oocyte recovery

In this study a total of 2117 oocytes were retrieved using slicing technique with a recovery rate of 4.91 oocytes per ovary which was different from Wahid *et. al.* (1992) and Datta *et. al.* (1993) used slicing technique and retrieved 6.4 and 7.36 oocytes per ovary. The variation observed could be due to the influence of reproductive status, endocrine profile, age of the animal and season at the time of slaughter. The percentage of A, B and C grade oocytes retrieved by slicing technique were 33.87 (717), 32.73 (693) and 33.40 (707) per cent, respectively. Wahid *et. al.* (1992) and Wani *et. al.* (2000) retrieved 74.90 and 54.30 percent A grade ovine oocytes by slicing technique. In this study slicing of ovary with Bard-Parker blade resulted in more cell debris which could have influenced the poor recovery rate of good quality oocytes (Wani *et. al.*, 1999). The higher percentage of C grade oocytes retrieved by slicing in this study might be due to the recovery of oocytes from all small diameter follicles which had not completed their growth and had a greater degree of atresia as indicated by Das *et. al.* (1996).

Oocyte maturation

The mean maturation percentage of A, B and C grade oocytes in the present study was 95.48 ± 0.63, 89.14 ± 1.17 and 77.94 ± 1.12, respectively. The higher maturation rate observed in both A and B grade oocytes than C grade concurred with the earlier report of Chauhan *et. al.* (1998). The higher maturation rate obtained in A and B grade oocytes compared to C grade in this study could be attributed to presence of cumulus cells surrounding the zona pellucida were linked to one another by gap junctions which permitted the transfer of nutrients and therefore played an important nutritive role during maturation as indicated by Sutovsky *et. al.* (1993). The overall maturation rate of oocytes in the present study was 87.5 ± 1.08 per cent. The result of this study was in the close agreement with the findings of Rao *et. al.* (2002) in sheep. However, higher maturation percentage of oocytes observed by Sun *et. al.* (1994) when compared to the present study might be due to the variation in the source and batch of serum used, source of oocytes and occurrence of cumulus expansion without oocyte maturation.

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