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Effect of collection methods on the recovery rate and *in vitro* maturation rate of ovine oocytes

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The number of good quality oocytes harvested from an ovary is an important aspect in the *in vitro* maturation and production of embryos. The ovaries of abattoir origin are the cheapest and most abundant source for large scale production of mature oocytes and domestic animal embryos. The oocytes from the abattoir ovaries can be harvested from 1–2 h (Pugh *et al.* 1991) to 3–4 h (Wani *et al.* 1999) without any detrimental effect on oocyte maturation. In the past, follicular dissection was employed for harvesting of ovine follicular oocytes and collection and *in vitro* maturation of oocytes have been reported in the recent years in sheep (Rangasamy *et al*. 2010) and goats (Wang *et al*. 2007).However, the recovery rate of oocytes varies with species and season of collection particularly in seasonal breeding species (Arangasamy *et al.* 2008, Nandi and Kumar 2008). Therefore, the present study was conducted to evaluate the recovery rate of oocytes by 3 oocyte collection techniques and to study *in vitro* maturation rate of oocytes harvested by slicing and puncture method, which is having meager literature in sheep during the non breeding season from the ovaries of abattoir origin.

Collection and processing of ovaries: Sheep ovaries were collected from local abattoirs during non breeding season in a thermos flask containing normal saline solution with 50µg gentamicin sulphate/ml. Extraneous tissues were trimmed from the ovaries and washed with 70% ethanol in the laboratory. A total of 90 ovaries were collected and 30 ovaries were subjected to each collection technique, viz. slicing, puncture and aspiration.

Oocyte collection and grading: The oocytes were collected from the ovaries by 3 methods, viz. slicing, puncture and aspiration.In slicing, all visible follicles on the ovary were sliced with a surgical blade in a petri-dish and observed under stereo zoom microscope. In puncture, visible follicles on the surface of ovary were punctured with 18-gauze needle in the petri-dish and observed under stereo zoom microscope. In aspiration, the follicular fluid from visible follicles was aspirated through a sterile 18-gauze needle attached to a 5 ml syringe and aspirated content was observed under stereo-zoom microscope for the presence of oocytes. Oocytes were grouped into good, fair and poor according to the character of cumulus cells (Pawshe *et al.* 1994) and total number of oocytes along with good, fair and poor oocytes was recorded for each ovary.

In vitro *maturation:* The usable oocytes collected by slicing (75) and puncture (141) method were matured separately. The oocytes were washed twice in maturation medium consisting of TCM-199 supplemented with 10% fetal calf serum, 10µg follicle stimulating hormone / ml and 50µg gentamicin sulphate / ml. Then, 10–15 oocytes were transferred to 35 mm petri-dish containing 2 ml of maturation medium equilibrated for 2 h in a CO_2 incubator and were cultured in 5% CO_2 in humidified air at 38.5° C in CO_2 incubator for 24 h. At the end of incubation period, the oocytes were evaluated for maturation status as per Kobayashi *et al*. (1994) under inverted microscope (200×magnifications) and expressed as the percentage of oocytes with degree 2 (D2) cumulus cell expansion. However, the matured oocytes were also examined for emission of first polar body after detachment of CCs by vortexing under inverted microscope.

Statistical analysis: The data were analyzed by paired T-test using statistical software SPSS version-17. All the data are expressed as mean±SEM. The level of significance was set at $P<0.05$.

The number of good, usable and total oocytes collected per ovary by slicing and puncture was significantly higher ($P< 0.05$) than the aspiration technique (Table 1). Comparatively, slicing method yielded higher number of fair, usable and total oocytes than the other 2 methods and poor

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Table 1. Effect of oocyte collection techniques on oocyte recovery rate

Collection method	Sample size (n)	Number of oocytes recovered (mean±SE)				
		Good	Fair	Poor	Total	Usable
Slicing	30	3.4[a]±0.22	1.87[a]±0.10	1.77[a]±0.12	7.03[a]±0.31	5.27[a]±0.28
Puncture	30	3.4[a]±0.24	1.83[a]±0.14	1.60[a]±0.13	6.83[a]±0.30	5.23[a]±0.26
Aspiration	30	1.67[b]±0.19	1.66[a]±0.14	1.36[a]±0.16	4.36[b]±0.34	3.03[b]±0.27

Means within the column with different superscripts (a, b) differ significantly at P<0.05.

oocytes recovered were lowest for aspiration and highest for slicing method of oocyte collection. However, the recovery rate of good quality oocytes per ovary was equal for slicing and punctures method.

Our study showed significantly higher recovery rate by slicing (7.03) and puncture (6.83) methods than aspiration (4.36) method. Slicing and puncture of the ovaries yielded a higher number of oocytes per ovary compared to aspiration (Wang *et al.* 2007) which supports the present study. In accordance of the present results, the higher number of good quality oocytes was also recovered using slicing (5.2) and puncture (5.2) method compared to aspiration (4.4) from sheep ovaries (Wani *et al.* 2000). Slicing yielded significantly more oocytes as well as good quality oocytes per ovary than puncture and aspiration technique in buffalo (Jamil *et al.* 2008) which is in line with the findings of current study. Significantly higher recovery rate of total oocytes per ovary for aspiration compared to puncture or slicing technique (Pawshe *et al.* 1994, Sherazi *et al.* 2005) and higher recovery rate of good quality oocytes by aspiration compared to slicing technique in pre-pubertal goats (Martino *et al.* 1994) was in contrast to our study. However, the higher recovery of good quality oocytes by slicing than aspiration (Sherazi *et al*. 2005, Pawshe *et al*. 1994) was in accordance with the present study. The recovery rate of good quality oocytes was lower for slicing compared to aspiration technique from pre-pubertal goats. Using slicing method, Rangasamy *et al.* (2010) recovered 4.5 oocytes and 2.7 culturable oocytes in sheep, and Mistry *et al.* (2010) recovered an average of 3.09 oocytes / ovary in buffalo. The difference in the number of oocytes recovered per ovary in the present study from the previous reports may be due to difference in season of sample collection, species and age of the animal as significantly higher recovery rates of good and total number of oocytes was recoded in breeding season than in non-breeding season in sheep (Nandi and Kumar 2008).

The maturation rate of oocytes collected by slicing and puncture was 62.66 and 71.63%, respectively, which was higher for puncture method than in slicing technique. Wang *et al.* (2007) reported the maturation rate of 60.8 and 84.0% of oocytes in goats collected by puncture and slicing technique which is in close agreement with our results. In prepubertal goats (Martino *et al.* 1994), the maturation rate obtained for slicing (56.8%) was lower than that obtained for dissection and aspiration (69.3 and 72.0%, respectively), which was in accordance with the present results. However, our results are in contrast to the findings of Wani *et al.* (2000), who found no significant difference in the maturation rate of oocytes in sheep collected by different collection methods. Similarly, Nowshari (2005) found no difference in the proportion of metaphase II oocytes harvested by aspiration and puncture (39 and 46%) in camel. The lower maturation rate may be due to more preantral oocytes collected by slicing method than by puncture as preantral oocytes have low maturation rate compared with antral oocytes (Izquierdo *et al.* 2002).

Table 2. Effect of collection methods on *in vitro* maturation rate

Collection method	Total oocytes	Degree of cumulus cell expansion (%)		
		DO	D1	D2 (mature)
Slicing	75	8 (10.67%)	18 (24.00%)	47 (62.66%)
Puncture	141	11 (7.8%)	29 (20.56%)	101 (71.63%)

Our study concluded that slicing and puncture are better oocyte collection methods from ovaries of abattoir origin in ovine. Although, slicing method gives the highest yield in terms of number of oocytes recovered per ovary of abattoir origin but the puncture method is more preferable oocyte collection technique - as it has the advantage that it produces less debris in the collection medium and makes oocyte collection and examination easy compared to slicing technique and gives higher maturation rate compared to slicing technique.

SUMMARY

Ovaries of slaughtered ewes were collected from abattoirs in normal saline solution containing 50µg gentamicin sulphate / ml to study the effect of collection methods, viz. slicing, puncture and aspiration on the recovery rate and *in vitro* maturation rate of oocytes. The number of good, usable and total oocytes collected per ovary by slicing (3.4, 5.27 and 7.03) and puncture (3.4, 5.23 and 6.83) was significantly higher than the aspiration (1.67, 3.03 and 4.36) technique. Comparatively, slicing method yielded higher number of usable and total oocytes than the other 2 methods and poor oocytes recovered were lowest for aspiration and highest for slicing method. However, recovery rate of good quality

oocytes was equal for slicing and puncture (3.4) method. The usable oocytes collected by slicing and puncture were matured *in vitro* , and maturation rate of oocytes collected by slicing and puncture was 62.66% and 71.63% respectively. The maturation rate was higher for oocytes retrieved by puncture method than slicing method. Thus, it was concluded that puncture technique is better oocyte collection method for ovaries of abattoir origin in ovine.

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