EFFECT OF RETRIEVAL TECHNIQUES ON THE QUALITY OF ABATTOIR OVARIAN FOLLICULAR OOCYTES IN ASSAM HILL GOATS

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ABSTRACT

The oocytes recovered (n=2539) from the abattoir collected goat ovaries (n=712) by aspiration, slicing and puncture techniques were washed 4-5 times in a washing medium (36 ml TCM-199, 4 ml Foetal Bovine Serum and 200 µl Gentamicin) and were graded as A, B and C on the basis of their cumulus cell layers surrounding the zona pellucida. The mean recovery rate of oocytes per ovary using slicing technique was higher (4.21±0.08, $p<0.05$) compared to aspiration (3.43±0.06) and puncture (3.24±0.08) techniques. The recovery of good quality oocytes (Grade A+B) was higher ($p<0.05$) in puncture (73.9±0.9%) than in aspiration (66.3±0.7%) and slicing (64.8±0.9%) technique. It can be concluded that puncture technique was most effective for the retrieval of good quality goat oocytes, whereas, the slicing technique yielded highest oocyte recovery rate.

Keywords: Abattoir, Goat, Oocyte, Recovery rate, Slicing

INTRODUCTION

In vitro embryo production in goat provides a source of low cost embryos for transfer in programme like MOET. The recovery of follicular oocytes from abattoir ovaries using different techniques could constitute the basis of production of large number of embryos *in vitro* through the processes of *in vitro* maturation and fertilization. The current study aimed to investigate the effect of different retrieval techniques on the oocyte recovery rate and quality of follicular oocytes.

MATERIALS AND METHODS

Goat ovaries were collected from local abattoirs as soon as possible after the animals were slaughtered. Ovaries were transported to the laboratory in a thermos flask containing warm (37^0C) normal saline solution with antibiotic. In laboratory, the extraneous ovarian tissue was removed and ovaries were washed 3-4 times with saline solution.

Ovarian oocytes were recovered immediately after washing by aspiration, slicing and puncture techniques. In aspiration technique, washing medium (1 ml) was taken in a 5 ml disposable syringe attached to a 18G needle and visible medium size follicles were punctured at the base and the contents of the follicle were aspirated out into the syringe. After aspiration of all the follicles, the contents of the syringe containing the oocytes was placed in a watch-glass and examined under a stereo-zoom microscope to ascertain the presence of oocytes. In slicing and puncture technique, ovaries were placed in a petri dish containing 5 ml washing medium and held with the help of a pair of forceps. Thereafter, in slicing procedure, incisions were given along the whole ovarian surface using a scalpel blade, and in puncture technique, the whole ovarian surface was punctured with an 18G needle.

A washing medium (TCM-199, 36 ml; Foetal Bovine Serum, 4ml; L-glutamine, 0.004g and Gentamicin, 200 µl; mixed ingredients were filtered using 0.22 µm syringe filter, kept overnight in a CO_2

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Table 1: Recovery rate (Mean±SE) of different grades of goat follicular oocytes from abattoir ovaries using different oocyte recovery techniques

Technique	Ovary (n)	Observation (n)	Oocyte recovered (n)	Oocyte recovery rate/ovary
Aspiration	338	28	1147	3.43±0.06[b]
Slicing	198	19	829	4.21±0.08[a]
Puncture	176	15	563	3.24±0.08[b]
Oocyte grade, % Recovery rate				
	A	B	A+B	C
Aspiration	43.7±0.7[a]	22.5±0.7[c]	66.3±0.7[b]	33.7±0.7[a]
Slicing	34.7±0.8[b]	30.1±0.5[b]	64.8±0.9[b]	35.2±0.9[a]
Puncture	33.4±0.6[b]	40.3±0.6[a]	73.9±0.9[a]	26.1±0.9[b]

[a vs b]$p<0.05$, within a column for a parameter

incubator maintaining 5% CO_2 at 38.5^{0}C with 90-95% relative humidity) was used to make the cumulus-oocyte complexes (COCs) free from debris.

The recovered ovarian oocytes were searched under a stereo-zoom microscope and were collected with the help of a micropipette. The oocytes were washed 4-5 times in the washing medium and were classified based upon the number of layer of cumulus cells adhered to the zona pellucida. The grade A, B or C oocytes were surrounded by ≥4 complete, 2-3 complete or 1 complete/incomplete layer of cumulus cells adhered to the zona pellucida.

RESULTS AND DISCUSSION

The oocyte recovery rate obtained in the present study by aspiration (3.43±0.06), slicing (4.21±0.08) and puncture (3.24±0.08) technique (Table 1), was comparable to previous studies in goats (Hoque *et al*., 2011 and John *et al*., 2015), whereas others had lower recovery rate by aspiration and higher recovery rate by puncture technique (Wang *et al*., 2007). Furthermore, the mean oocyte recovery rate was higher ($p<0.05$) in slicing compared to aspiration and puncture techniques, while results were similar ($p>0.05$) between aspiration and puncture techniques (Table 1). Others also had higher oocyte recovery rate per ovary by slicing technique in goats (Wang *et al*., 2007 and John *et al*., 2015). The higher recovery rate of cumulus-oocyte complexes per ovary in slicing could be due to slicing of oocytes from the surface follicles as well as from the deeper cortical stroma, whereas, in puncture and aspiration techniques oocytes are released from surface follicles alone (Das *et al*., 1996).

The recovery rate of different grades of follicular oocytes varied ($p<0.05$) between techniques (Table 1). The mean recovery rate of good quality oocytes (Grade A+B) was higher ($p<0.05$) in puncture than in aspiration and slicing technique (Table 1). This finding was in agreement with previous studies in goat (Wang *et al*., 2007 and John *et al*., 2015). However, others obtained comparatively low percentage of good quality oocytes by puncture than the present study (Pawshe *et al*., 1994). The higher recovery rate of good quality oocytes (Grade A + Grade B) by puncture technique could be due to the feasibility of obtaining oocytes covered with more layers of cumulus cells, while the recovery process by aspiration or slicing could lead to disruption of surrounding cumulus cells layers around the oocyte during the process of aspiration or slicing. The lowest percentage of good quality oocytes obtained by slicing technique might be due to damage caused to the surrounding layers of cumulus cells around the oocytes by the blade used during slicing.

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