

Comparative efficiency of different oocytes recovery methods from cattle ovaries

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Many workers have recovered oocytes either from live animals or ovaries collected from recently slaughtered animals, and the oocyte recovery rate of acceptable quality oocytes varies in different recovery methods (Viana *et al.* 2004, Yang *et al.* 2005, Cox and Alfaro 2007, Ireland *et al.* 2007). The present study was conducted to compare the efficiency of different oocyte recovery methods from cattle ovaries collected from slaughterhouse.

Standard chemicals, media, disposable filters were used for the present experiment. Cattle ovaries were collected from Kolkata slaughterhouse and were transported within 3–4 h to the laboratory in 0.9% saline at 30–35°C. Follicular oocytes were collected from ovaries adopting 3 different methods. In aspiration method, oocytes were collected from the visible follicles of ovaries with a 19-gauge needle attached to a 5 ml disposable plastic syringe. The aspiration medium consisted of TCM-199 supplemented with 5% fetal bovine serum (FBS) and Dulbecco's phosphate buffered saline (DPBS) supplemented with 0.3% bovine serum albumin (BSA). In puncturing method follicles were punctured with 19-gauge needle the ovaries in the collection media (TCM-199 supplemented with 5% FBS). In slicing method, ovaries were sliced (1–2 mm thickness) with BP blade keeping in the collection media.

The oocytes were searched and collected from the collection media under the stereo-zoom microscope. The oocytes were graded on the basis of the presence of cumulus cells as grade A oocytes having 4–5 layers of cumulus cells,

grade B having 2–3 layers of cumulus cells and grade C having 1–2 layers of irregular cumulus cells. After grading, oocytes were washed thoroughly (5–6 times) in the washing media (TCM-199 supplemented with 5% FBS) and cryopreserved in liquid nitrogen by vitrification. Observations were recorded on different grades of oocytes in different recovery methods. The data were analyzed by analysis of variance (Snedecor and Cochran 1967).

In the present study, 417 cattle ovaries were collected and total 586 oocytes were recovered in different grades (Table 1). In the puncturing method, highest percentage of A grade oocytes was recovered but in slicing method highest percentage of B grade oocytes was recovered. In aspiration method, maximum number of C grade oocytes were recovered, which is not significantly different from the other 2 methods. The recovery rate of denuded oocytes (i.e. D grade) was the highest in slicing method. Therefore, slicing of ovary is comparatively the best method for oocyte recovery from cattle ovaries followed by aspiration and puncturing methods. Sharma (1990) reported that oocytes recovered from isolated follicles were better over aspiration and slicing techniques in buffaloes. Gordon and Lu (1990) reported oocyte recovery rate up to 10/ovary in exotic breeds. As compared to cattle, very low recovery rate (0.43/ovary) was obtained in buffalo by Totey *et al.* (1992). Arav (2001) by using transillumination technique, obtained 36% oocyte recovery rate from ovaries collected at slaughter. From live animal through OPU, Manik *et al.* (2003) recovered overall 59%

Table 1. Oocytes recovered from ovaries by 3 different methods

Methods	Total no. of ovaries	Different grades of oocytes collected				Total No. of oocytes	No. of oocytes per ovary
		A (4–5 layers)	B (2–3 layers)	C (1–2 layers)	D (No layers)		
Aspiration	171	48 (20.69)	58 (25.00)	76 (32.76)	50 (21.55)	232	1.36
Puncturing	107	31 (27.93)	28 (25.23)	35 (31.53)	17 (15.32)	111	1.04
Slicing	139	56 (23.05)	66 (27.16)	74 (30.45)	47 (42.34)	243	1.75

*The number under parenthesis indicates the percentage.

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oocytes (range 35–79%) and of these 32% were A grade oocytes. The highest oocyte recovery rate (38.8%) in small

livestock animal (sheep) was reported in slicing method by Datta *et al.* (1993). Cox and Alfaro (2007) collected oocytes through laparoscopic aided ovum pick-up from goats and sheep to assess the current procedures used in cattle for production of IVP embryos and obtained 81.2% and 85.2% in goat and sheep respectively. The oocytes recovered once in a week from Gir breed through ultrasonographically guided aspiration method was 8.9 ± 0.8 , which was higher than when aspirated twice in a week (7.0 ± 0.7) (Viana *et al.* 2004). Yang *et al.* (2005) collected oocytes from F1 hybrid cattle and their parental lines and reported the recovery rate of 7.8 ± 0.5 . After superovulation the number of oocytes recovered was 4.7 ± 0.7 in low follicle number group animals and 10.6 ± 2.7 in high follicle number group animals (Ireland *et al.* 2007). It may be concluded from the above study that the slicing of ovary was the best method for oocyte recovery from the slaughterhouse ovaries, followed by aspiration and puncturing method.

SUMMARY

The present study was undertaken in cattle to study the efficiency of 3 different methods of oocyte recovery. Slaughterhouse ovaries were collected and the oocytes were collected from the ovaries by aspiration, puncturing and slicing methods. Total 417 cattle ovaries were collected and 586 oocytes were recovered in different grades. The maximum number (27.93%) of A grade oocytes were recovered by puncturing method followed by slicing (23.05%) and aspiration method (20.69%). Slicing method showed the highest recovery rate per ovary i.e. 1.75 whereas puncturing method showed the lowest recovery rate per ovary (1.04). It can be concluded that slicing of ovary is the best oocyte recovery method.

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