COMPARATIVE OVARIAN BIOMETRY AND OOCYTE RETRIEVAL METHODS IN PIG

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Reproductive success is central to livestock production. Advancement in porcine reproduction has become more important for developing novel biotechnology, as well as for genetic improvement of livestock (Choi *et al.*, 2008). *In vitro* reproductive techniques are powerful tools for studying physiology of maturation, fertilization, development of pre-implantation embryos and increasing production as it gives access to micromanipulation of embryos (Ramsingh *et al*., 2013). For successful *in vitro* production (IVP) of embryos, evaluation of ovaries and efficient collection and grading of oocytes is very important (Islam *et al*., 2007). Large number of oocyte is a prerequisite factor for such studies. However, there has been a limitation to retrieve sufficient number of the oocytes. The total number of oocytes obtained per ovary varies with different collection methods. Therefore, for efficient IVP of embryo from slaughterhouse-sourced ovaries it is necessary to develop a suitable technique that can enhance the oocyte recovery rate. The present study was undertaken for evaluation of slaughterhouse-sourced porcine ovaries for IVP of embryos and relative efficiency of oocyte retrieval methods.

Ovaries of adult crossbred sows, used for the present study, were sourced from organized slaughterhouse (R&D Pork Processing Plant, ICAR-NRC on Pig, Rani, Guwahati) and unorganized slaughterhouses located at Rani, Guwahati. The reported experiment was approved by Institutional Animal Ethics Committee. A total of 178 numbers of ovaries were collected immediately after slaughter and transported to the laboratory within 2 hr at room temperature in normal saline solution (NSS) containing penicillin (100 IU/ml) and streptomycin (100 µg/ml). The ovaries were washed three times with clean water and finally washed in NSS containing penicillin and streptomycin.

Morphometric measurements, e.g. length and width of the ovaries, were measured with a Vernier caliper. The measure from the anterior to posterior end of the ovary was considered as the length, and medial to lateral border of the ovary was considered as width. Numbers of visible surface follicle, corpus luteum and abnormalities (if any) were recorded.

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The oocytes were collected aseptically from the ovaries by following two methods (Fig. 1):

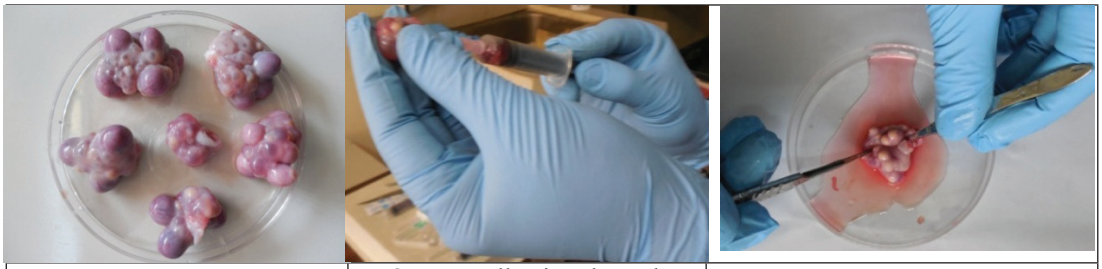

Fig. 1: Pig ovary	Oocyte collection through aspiration	Oocyte collection through slicing

1. Aspiration: The visible follicles (2-8 mm in diameter) present on the surface of the ovary were aspirated with 18G needle fixed to a disposable syringe containing 1 ml of tissue culture medium (TCM) 199 (Sigma). The collected pig oocytes were placed in a 60 mm petridish (Nunc) containing TCM 199 and searched under steriozoom microscope for grading.

2. Slicing: The ovaries were held firmly with the help of a forceps in a sterile glass petridish containing 5 ml of warm (37 ˚C) TCM 199. The ovaries were sliced into possible small sections with a BP blade fixed to a handle. The oocytes containing TCM 199 media were transferred to a searching dish and observed under steriozoom microscope for grading of the oocytes.

Grading of oocytes:

Oocytes were classified on the basis of cumulus layers as follows:

Grade A: Compact, multi-layered (more than three) cumulus with a homogenous cytoplasm

Grade B: Compact cumulus consisting of two to three layers of cells, with a homogenous ooplasm

Grade C: Less compact cumulus, with an irregular ooplasm, containing dark clusters in ooplasm

Grade D: Depleted of complex cells or an expanding cumulus, irregular ooplasm and jelly-like matrix.

Statistical analysis:

Data were analysed using SPSS (ver. 16.0). Morphometric measurements are presented in Mean±SE. Harvest of graded oocytes is presented in percentage. The percentage values were converted using arcsine transformation before two-sample T-test/ANOVA**.**

Biometry and follicular parameters:

The biometry and follicular parameters of ovaries collected from organized and unorganized-slaughterhouses are presented in Table 1. Average length and width of the ovaries sourced from organized slaughterhouse were higher than the ovaries sourced from unorganized slaughterhouses.

Similarly, average numbers of surface and cystic follicles were higher for organized slaughterhouse-sourced ovaries. Number of corpus luteum (CL) was higher for unorganized slaughterhouse-sourced ovaries. However, these differences were not significant. Significant differences ($P<0.05$) in morphometric traits of the porcine ovaries was observed by Naskar *et al.,* (2015) based on source. Number of surface follicles was reported to be significantly higher in unorganized slaughterhouse-sourced ovaries ($P<0.05$) (Naskar *et al.,* 2015). Further, number of CL was higher for porcine ovaries collected from organized slaughterhouses (Naskar *et al.,* 2015). The variation observed in the present study compared to other reports may be due to the fact that number of surface follicles, cystic follicles and CL present in an ovary is influenced by many factors, like breed, stage of reproductive life and reproductive health. Biometry of ovaries and follicular parameters further indicates that female pigs slaughtered in organized slaughterhouses are likely beyond third or fourth farrowing where reproductive worth has already been realized that is common for organized production system. On the other hand, female pigs slaughtered in unorganized slaughterhouses are likely to be slaughtered before third or fourth farrowing, even at prepubertal stage (Dyck and Swierstra, 1983; Bartol *et al.,* 1993; Naskar *et al.,* 2015).

Oocyte recovery and grading:

Average number of oocytes recovered per ovary by slicing (12.93±1.49) was significantly higher ($P<0.01$) than aspiration (6.36±1.02). Overall percentage of different grade of oocytes, namely 'A', 'B', 'C' and 'D', recovered from 178 numbers of ovaries used in our experiment are presented in Table 2. It is important to note that 'A' grade oocytes are the best source material for applications of assisted reproductive technologies (ART). Our study indicates that aspiration method yields more 'A' grade oocytes than slicing ($P<0.01$). On the other hand, slicing method yields more 'B', 'C' ($P<0.01$) and 'D' ($P<0.05$) grade oocytes. These might be due to the fact that oocytes are recovered from mature surface follicle only in aspiration as against predominant retrieval of oocytes from core of cortex of the ovary in slicing which may not have developed enough and thus unfit for ART. Similar results were obtained by Wani *et al*. (2000), Wang *et al.* (2007) and Zeinoaldini *et al.* (2013).

Biometry and follicular parameters of porcine ovaries collected from organized and unorganized-slaughterhouses are reported in the present study. Relative comparison of oocyte retrieval methods reveals that aspiration yields higher percentage of superior grade oocytes (A) suitable for applications of ART. Further, our study reveals that ovaries sourced from unorganized slaughterhouses can also be used for ART, and use of aspiration method may yield better quality oocytes.

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Table 1: Biometry and follicular parameters of ovaries collected from organized and unorganized-slaughterhouses

Source (n = no.)	Length (mm)	Width (mm)	Follicle (no.)	CL (no.)	Cystic follicle (no.)
Unorg. Slaughterhouse (n=103)	26.74 ± 0.79	18.84±0.55	14.26±1.17	8.43±0.74	0.35±0.19
Org. Slaughterhouse (n=75)	30.50±1.50	21.70±1.27	19.70±4.25	5.70±1.76	1.40±0.98

Table 2: Recovery rate of different grade of oocytes by different methods of oocyte collection

Oocyte collection method	Grade of oocyte (in percentage)			
	A	B	C	D
Aspiration	65.21±5.71[a]	21.32±4.99[a]	3.03±1.63[a]	10.44±2.27[a]
Slicing	34.43±4.08[b]	23.37±2.51[a]	15.16±2.01[b]	27.04±4.65[b]

Means with different superscript within a column differ significantly ($P<0.01$ for A and C grade; $P<0.05$ for D grade)