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Morphometric study of ovary and rate of recovery of oocyte from medium size follicle by aspiration technique in cattle

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Abstract
A research work was conducted to study the ovarian biometry, follicular biometry and recovery percentage of oocyte from the ovaries of slaughter house. A total of 209 cattle ovaries obtained from the slaughter house soon after sacrifice were utilized during the study period and out of this 43 ovaries were utilized randomly for ovarian biometry and 166 ovaries were utilized for follicular biometry. Significantly higher length, width, thickness and weight were recorded in ovary with CL than that without CL. The number of large, medium and small follicles was more in ovary without CL than with CL group. The mean number of medium size follicles was significantly ($P<0.01$) higher in ovary without CL (6.32±0.75) than with CL (3.33±0.18). The recovery rates of grade A (47.58%) and B (37.42%) oocytes were higher than that of grade C (8.82%) and D (6.12%) by aspiration method.

Keywords: Morphometry Biometry, Aspiration, ovary, cattle

Introduction
In-vitro production (IVP) of embryos is currently the central focus in livestock industry. Proper selection of oocyte is very important for successful embryo production. For any successful IVP programme in cattle and other livestock, artificial removal of cumulus oocyte-complexes (COC) from mature follicles, culturing and maturing them is a primary requirement where the optional rate of embryo production in-vitro can be attained by selecting ovaries and follicles, which provides oocyte to undergo maturation, suceesful fertilization, and *in-vitro* development. Selection and grouping of oocytes according to morphological appearance helped to improve maturation ability (Leibfried-Rutledge and First, 1979; Staigmiller *et al.* 1988*;* Nagano *et al.* 2006; Wang and Sun, 2006) [12, 15, 19]. Extensive research on in-vitro maturation (IVM), in-vitro fertilization (IVF) and in-vitro culture of the resulting zygote in cattle has been reported by (Agrawal *et al.,* 1995) but limited information has been reported on the evalution of cattle ovaries or method for the efficient collection and grading of oocyte. The morphology of cumulus investment is commonly used as selection criteria prior to IVM, which greatly influence to the maturity of oocytes, COCs matured in-vitro and subsequent studies, further confirmed the observation. Denuded oocyte with few cumulus cells are usually rejected because of their low capacity of fertilization and in-vitro development (Rahman *et al.,* 2008) [16]. Generally, Selection was based on visual assessment of follicular cells-cumulus-oocytes-complexes (COC) and corona radiata via their thickness and compactness (Nagano *et al.,* 2006; Wang and Sun, 2006) [15, 19]. The morphology of cattle ovaries in relation to follicular growth, corpus luteum, with the view of in-vitro recovery, quality and maturation of oocytes still need more investigation where the quantitative aspects of follicular growth have been studied in cattle (Singh and G.P., 2000) [18] and sheep (Mohammadpour, 2007) [14]. The *in-vitro* embryo production procedures developed for cattle have been improved significantly, but many factor influencing their efficiency still need to be investigated. Although there appeared many reports on successful pregnancies out of *in-vitro* produced embryos it had been felt necessary to conduct more studies with a view to improving the *in-vitro* development rates of cattle oocyte. Slaughtering of quality cows was very common in local situations. The present research work has been undertaken for collection and evaluation of slaughterhouse ovaries, follicle and recovery percentage of oocyte by aspiration technique to evaluate the effect of follicles, corpus luteum on the morphology of cattle ovaries and would certainly help to prevent the loss of these quality oocytes.

Materials and Methods

Collection of Ovaries

Cattle ovaries were collected from the local abattoirs as early as possible after the animals were slaughtered and carried to the laboratory in a thermos flask containing warmed (37 ^{0}C) normal saline solution (N.S.S.; 9%) with antibiotics (transportation medium). In the laboratory extra tissues adhering to the ovaries were removed with scissors and washed 3-4 times in transportation medium and finally washed in Dulbecco's phosphate buffer saline (DPBS). The ovaries were subjected to biometric observations that included recording of weight, number of visible follicles and corpus luteum.

Morphometric analysis of Ovaries

(a) Measurement of weight and size of the ovary

Fig 1: Biometric measurements of Ovary and follicles
(a) Length of ovary (b) Width of ovary (c) Thickness of ovary (d) Diameter of follicle

The ovaries were allocated into two groups: ovary with corpus luteum (CL) and ovary without corpus luteum (CL). In the laboratory, excessive tissues attached to the ovaries were carefully trimmed off and ovaries were weighed using an electronic balance. The length (distance between anterior and posterior pole), width (distance between medial and lateral surface) and thickness (distance between attached and free border) of the ovaries were individually measured using Vernier calipers.

(b) Determination of follicular population

The ovaries were washed in the transportation medium. For each ovary, visible follicles were counted and follicular diameter was measured using Vernier calipers. Follicles were classified based on diameter into three categories: small (<3mm), medium (3 to 8 mm) and large (>8 mm).

Recovery of Oocytes

The oocytes were collected from the medium size (3-8 mm) follicles by aspiration technique. One milliliter of sterile aspiration medium was taken in a 5 ml disposable syringe attached to a 18-gauge needle. With the help of the needle medium size follicles were punctured through stroma at the base and then the content of the follicle was aspirated out into the syringe. After aspiration of all the follicles, the content of the syringe containing the oocytes was placed in a watch glass and examined under a stereozoom microscope to ascertain the presence of oocyte.

Fig 2: Aspiration of Oocyte from the medium size follicles

The aspiration medium was prepared using TCM-199 as a base medium along with additives. After mixing of the ingredients, the medium was filtered using 0.2 µm syringe filter and incubated in a CO_2 incubator maintaining 38.5 ^{0}C with 90-95 per cent relative humidity for 2 hours before use.

Statistical Analysis

Data obtained in the present experiment were analyzed statistically by SAS Enterprise Guide 4.3.

Experimental Findings

Morphometry of Ovary

In the present study mean weight, length, width and thickness of ovary with and without corpus luteum (CL) are presented in Table 1. The mean weight of the ovary with and without corpus luteum (CL) was recorded to be as 3.26±0.04 and 2.05±0.23 g respectively which differed significantly (P<0.01). The length, width and thickness were 2.40±0.12, 1.76±0.12 and 1.23±0.07 cm, and 2.04±0.08, 1.44±0.08 and 1.00±0.05 cm in ovary with CL and without CL respectively. The mean weight of the ovary with CL was significantly (P<0.01) higher than that of the ovary without CL. The mean length, width and thickness of the ovary with CL were significantly (P<0.05) higher than the ovary without CL.

The mean number of large, medium and small follicles recorded per ovary in the present study was 0.67±0.13, 3.33±0.18, 3.93±0.32 and 0.88±0.07, 6.32±0.75, 4.67±0.31 in ovary with CL and without CL respectively (Table 2).

The t-value revealed that the mean number of medium size follicle per ovary differed significantly (P<0.01) between ovary with CL and ovary without CL. However, there was no significant difference in mean number of large and small follicles between ovary with CL and without CL.

Rate of Recovery of different Grades of Oocytes

The recovery rate of A, B, C and D grades of oocyte using aspiration method was found to be 47.58, 37.42, 8.82 and 6.12 per cent respectively. The chi-square value revealed significant difference (P<0.01) among the recovery rates of different grades of oocytes by aspiration method (Table 3 and Figure 5).

Table 1: Morphometry of Cattle Ovary collected from Slaughter house

Measurement	Types of ovary		t-value
	Ovary with CL (13) (Mean ± SE)	Ovary without CL (30) (Mean ± SE)	
Weight (g)	3.26±0.04	2.05±0.23	2.78**
Length (Cm)	2.40±0.12	2.04±0.08	2.38*
Width (Cm)	1.76±0.12	1.44±0.08	2.10*
Thickness (Cm)	1.23±0.07	1.00±0.05	2.64*

Figures in the parenthesis indicate number of observation
*P<0.05
** P<0.01

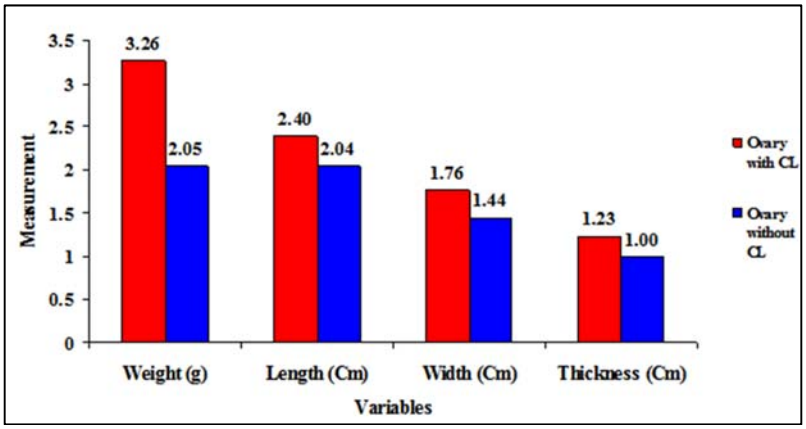

Fig 3: Morphometry of Cattle Ovary with and without CL

Table 2: Number (Mean ±SE) of different types of follicle per Ovary

Types of follicle	Types of ovary		t-value
	Ovary with CL (47)	Ovary without CL (119)	
Large	0.67±0.13	0.88±0.07	1.45 NS
Medium	3.33±0.18	6.32±0.75	3.84 **
Small	3.93±0.32	4.67±0.31	1.65 NS

Figures in the parentheses indicate number of observation.
NS Non-significant
** P<0.01

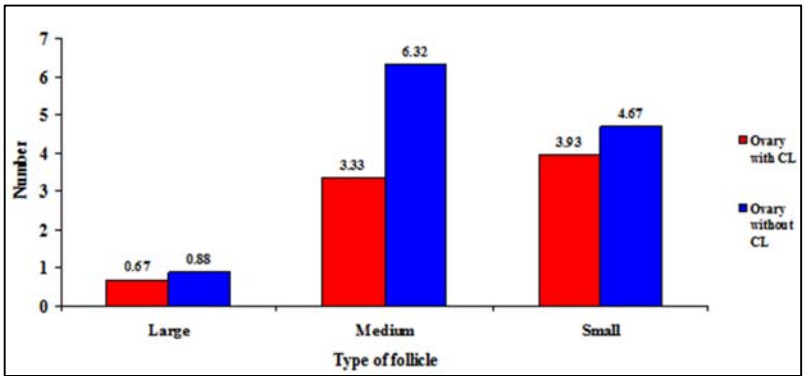

Fig 4: Recovery of mean number of different types of follicle per Ovary with and without CL

Table 3: Rate of recovery of different Grades of Oocyte by Aspiration method

Total number of ovary	Grade of oocyte	No of oocyte recovered	Rate (%) of oocyte recovered	Chi-square value
166	A	295	47.58	317.54**
	B	232	37.42	
	C	55	8.82	
	D	38	6.12	
	Overall	620		

** P<0.01

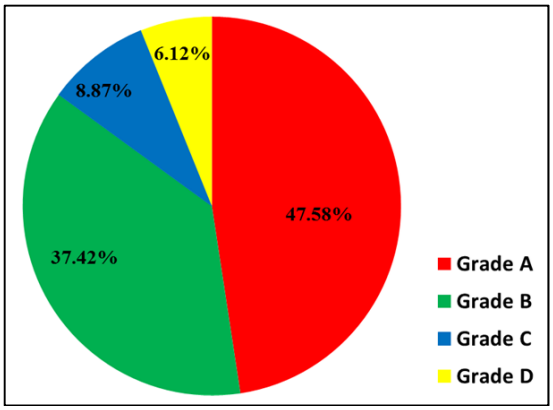

Fig 5: Rate of recovery of different Grades of Oocytes by Aspiration method

Discussion

Morphometry of Ovary

Results obtained in the present study indicated that morphometric measurements such as weight, length, width and thickness were greater in the ovary containing CL as compared to that without CL. As regards to the number of different types of ovarian follicle, it was observed that these two types of ovaries did not vary significantly in the number of large and small follicles per ovary. However, number of medium size follicle was significantly higher in ovary without CL as compared to that with CL. Corpus luteum which had been reported as an extra cellular material within the ovary having a definite span of its growth, maintenance and regression might be the cause of differences in length, width, thickness and the weight of the ovary containing it (Jablonka-Sharif *et al.,* 1993) [10]. The higher number of follicle in the ovary without CL as observed in the present study might be indicative of higher level of gonadotropins and steroid activity in it (Asad *et al.,* 2016) [3]. Lesser number of follicles in the CL containing ovary might be due to the inhibitory effect of CL on follicular growth (Hafez, 1993) [5]. The results of the present finding was comparable with that of Khandoker *et al.* (2011) [11] who working on buffalo ovaries reported that length, width and weight of the ovary increased significantly (P<0.05) due to presence of CL. The authors also observed that the number of aspirated follicles per ovary were significantly higher in ovaries without CL (6.78±0.18) than that with CL (4.09±0.26). Similar findings were also reported by Islam *et al.* (2007) [9] in case of goat.

Rate of recovery of different Grades of Oocytes

The recovery rate of A, B, C and D grade of oocytes using aspiration method was found to be 47.58, 37.42, 8.87 and 6.12 per cent respectively and there was significant difference (P<0.01) among the recovery rates of different grades of oocytes. The results obtained in the present study showed that recovery percentage of grade A and B oocytes was higher than that of grade C and D. This result was comparable with the findings of Ahmed *et al.* (2015) [2] who recorded higher percentage of grade A (38.77%) and B (27.02%) oocyte than that of grade C (18.13%) and D (16.08%) in bovine ovaries. Hussain (2011) [8] in yak ovaries also recorded higher recovery rate of grade A (45.45%) and B (40.41%) oocytes using aspiration method. On the other hand, Wang *et al.* (2007) [20] recorded much lower rate of recovery of grade A (21.1%) oocytes in cattle ovaries as compared to that observed in the present study. The discrepancy could be attributed to differences in the size of the ovary and follicle from which oocytes were recovered (Boni, 2012) [4]. Higher yield of good quality oocytes obtained by aspiration technique as observed in the present study might be due to the collection of oocytes only from medium size follicles (Hosek *et al.,* 1986) [7] using wide lobe of 18 gauze needle which might had reduced the damage of cumulus cell layers during collection (Hashimoto *et al.,* 1999). Also, it might be due to the recovery of oocytes from the ovaries of animal with better reproductive status and containing higher number of medium and active follicles from which the oocytes were retrieved (Mehmood *et al.,* 2011) [13].

Conclusion

Morphometric measurements such as length, width, thickness and weight were higher in the ovary containing CL but the number of follicles was higher in the ovary without CL with significantly higher proportion of medium follicles. The rates of recovery of grade A and B oocytes were higher than that of grade C and D oocytes following aspiration method of bovine ovaries

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