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

M Bhajoni, D Bhuyan, RK Biswas and DJ Dutta

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, successful 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 evaluation 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 °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

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.

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

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Types of ovary</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ovary with</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CL (13) (Mean ± SE)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ovary without CL (30) (Mean ± SE)</td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>3.26±0.04</td>
<td>2.05±0.23</td>
</tr>
<tr>
<td>Length (Cm)</td>
<td>2.40±0.12</td>
<td>2.04±0.08</td>
</tr>
<tr>
<td>Width (Cm)</td>
<td>1.76±0.12</td>
<td>1.44±0.08</td>
</tr>
<tr>
<td>Thickness (Cm)</td>
<td>1.23±0.07</td>
<td>1.00±0.05</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Types of follicle</th>
<th>Types of ovary</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ovary with CL (47)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ovary without CL (119)</td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>0.67±0.13</td>
<td>0.88±0.07</td>
</tr>
<tr>
<td>Medium</td>
<td>3.33±0.18</td>
<td>6.32±0.75</td>
</tr>
<tr>
<td>Small</td>
<td>3.93±0.32</td>
<td>4.67±0.31</td>
</tr>
</tbody>
</table>

Figures in the parentheses indicate number of observation.
NS Non-significant
** P<0.01
Table 3: Rate of recovery of different Grades of Oocyte by Aspiration method

<table>
<thead>
<tr>
<th>Total number of ovary</th>
<th>Grade of oocyte</th>
<th>No of oocyte recovered</th>
<th>Rate (%) of oocyte recovered</th>
<th>Chi-square value</th>
</tr>
</thead>
<tbody>
<tr>
<td>166</td>
<td>A</td>
<td>295</td>
<td>47.58</td>
<td>317.54**</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>232</td>
<td>37.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>55</td>
<td>8.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>38</td>
<td>6.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>620</td>
<td></td>
<td>** P&lt;0.01</td>
</tr>
</tbody>
</table>

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 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.

Recovery rate of different Grades of Oocytes

<table>
<thead>
<tr>
<th>Aspiration method</th>
<th>Grade A</th>
<th>Grade B</th>
<th>Grade C</th>
<th>Grade D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of recovery</td>
<td>47.58%</td>
<td>37.42%</td>
<td>8.87%</td>
<td>6.12%</td>
</tr>
</tbody>
</table>

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.

Reference


