EVALUATION OF OOCYTE RETRIVAL METHODS FROM BUBALINE OVARIES

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ABSTRACT

A total of 900 buffalo ovaries obtained from slaughter house were utilized for certain morphometric observations. The length and width of buffalo ovary averaged 2.17±0.07 and 1.46±0.06 cm, respectively. Average number to observable follicles on the ovarian surface was 4.12±1.32 per ovary with an overall average of 2.68±0.91 oocytes per ovary. The oocytes were recovered from ovaries using three different methods i.e. follicle isolation, aspiration and slicing. Average number of oocytes recovered by slicing (3.09) was highest followed by follicle isolation (2.61) and by aspiration (1.89). Postaspiration slicing 'yielded 0.46 extra oocyte per ovary. Corpus luteum was observed on 32.0% of ovaries. The oocyte recovery was higher from ovaries having corpus luteum. Time taken to process individual ovary was 2.05, 10.25 and 3.35 min. for slicing, follicle isolation and aspiration, respectively. Though the aspiration method yielded least number of oocytes, it proved to be the most applicable method for IVM and IVF.

INTRODUCTION

Animal embryo biotechnology is gaining momentum day by day and has become multi-dimensional. Thus, it is imperative that large number of embryos of various developmental stages will be required by researchers, breeders and possibly by the clinicians for overall development of animal industry. The utilization of ova from slaughtered animals, assures a steady source of oocyte supply and most research workers utilize the abbatoir materials for IVF studies. Collection of optimum number of good quality culturable oocytes is one of the important factor, particularly in species like buffalo where the IVF procedure is still in the stage of experimentation. Various techniques are available for collecting the oocytes from ovary. The present investigation was made to study the comparative efficacy of three methods of oocyte collection from slaughter house buffalo ovaries.

MATERIAL AND METHODS

Buffalo ovaries were collected from Haldwani slaughter house and were transported to the laboratory in Dulbecco's phosphate buffer saline (DPBS) within 90 minutes of slaughter. The ovaries were washed thoroughly four times with DPBS and then two times with 60 per cent alcohol and then kept in DBPS for subjecting to certain morphological examination viz. length and width of ovary, number of observable follicles, presence of corpus lutea, etc. A total of 900 ovaries were collected from January to October 1998 and they were divided into three groups for collection of oocytes by slicing, follicle isolation and aspiration methods.

(a) Slicing: The ovaries were held firmly in a petriplate having washing medium and were sliced with a surgical scalpel. Pieces of ovarian stroma were transferred to fresh washing medium in another petriplate, rinsed and discarded. Oocytes were searched under stereoscopic microscope in the washing medium in two plates.

(b) Follicle Isolation: The ovaries were held properly and follicles were removed after dissecting and teasing the ovarian tissue with fine scalpel, scissors and needles. Follicles were
and morphometric average length and width to be 2.44 cm and 1.36 cm, respectively and those of Sane et al. (1964) whose estimates for these parameters were 2.94 cm and 1.38 cm, respectively. Values obtained in the present study are marginally lower, apparently because of non-selected nature of the samples.

The average number of follicles (2-8 mm) on surface of ovary was 4.12 ± 1.321. Corpus lutea was observed on 32 per cent of ovaries whereas 16.33 per cent ovaries were devoid of any surface follicle or corpus luteum.

The time taken for oocyte collection per ovary was recorded to be 2.05, 10.25 and 3.35 min. for slicing, follicle isolation and aspiration methods, respectively. As the ovarian stroma of buffalo ovary is hard and follicles are deeply embedded, the isolation of follicles is difficult and takes long time to isolate individual follicles. The oocyte recovery was observed as 73.41, 61.97 and 47.80 per cent by slicing, follicle isolation and aspiration, respectively with an overall recovery rate of 65.04 per cent.

The number of oocytes recovered per ovary averaged 3.09, 2.61 and 1.89 by slicing, follicle isolation and aspiration, respectively. Almost similar observation were recorded by Sharma (1990) and Jalla (1991). Post aspiration slicing provided additional 0.46 oocytes per ovary. The overall recovery averaged 2.68 ± 0.91 per ovary. (Table 1 a, b).

RESULTS AND DISCUSSION
The length and width of buffalo ovaries averaged 2.17 ± 0.074 cm and 1.46 ± 0.058 cm, respectively as compared to the earlier reports of Bhalla et al. (1964), who found average length and width to be 2.44 cm and 1.36 cm, respectively and those of Sane et al. (1964) whose estimates for these parameters were 2.94 cm and 1.38 cm, respectively. Values obtained in the present study are marginally lower, apparently because of non-selected nature of the samples.

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Table 1. Comparison of different methods of oocyte recovery and morphometric observations of buffalo ovaries.

<table>
<thead>
<tr>
<th>Method</th>
<th>No of % ovaries</th>
<th>Time (min/ov)</th>
<th>No of follicles observed</th>
<th>No of oocytes recovered</th>
<th>Oocytes recovered per ovary</th>
<th>% of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slicing</td>
<td>300</td>
<td>2.05</td>
<td>1260</td>
<td>925</td>
<td>3.09</td>
<td>73.41</td>
</tr>
<tr>
<td>Follicle isolation</td>
<td>300</td>
<td>10.25</td>
<td>1262</td>
<td>782</td>
<td>2.61</td>
<td>61.97</td>
</tr>
<tr>
<td>Aspiration</td>
<td>300</td>
<td>3.35</td>
<td>1186</td>
<td>567</td>
<td>1.89</td>
<td>47.80</td>
</tr>
<tr>
<td>Post-aspiration</td>
<td>(300)</td>
<td>-</td>
<td>-</td>
<td>138</td>
<td>0.46</td>
<td>11.63</td>
</tr>
<tr>
<td>Slicing</td>
<td>Total</td>
<td>900</td>
<td>3708</td>
<td>2412</td>
<td>2.68 ± 0.91</td>
<td>65.04</td>
</tr>
</tbody>
</table>

* Average number of observable follicles per ovary.
<table>
<thead>
<tr>
<th>No of ovaries</th>
<th>Length (cm)</th>
<th>Width (cm)</th>
<th>No. of ovary (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>900</td>
<td>2.17±0.074</td>
<td>1.46±0.058</td>
<td>With CL 32.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Smooth 16.33</td>
</tr>
</tbody>
</table>

In the present study the oocyte recovery is comparatively more than obtained by Totey et al. (1992) and Dutta (1994). The variation in the oocyte recovery may be due to different breeds of the buffalo native to various localities and their nutritional status. The seasons in which the oocytes were harvested by various workers are not known which may have greater influence on the yield of oocytes. Furthermore, the expertise of individuals in processing the ovaries and the load of handling number of the ovaries per day could have also affected the efficiency of recovery.

It was also observed that though the slicing was most easier but the presence of ovarian stroma debris and blood clots and fibrin shreds makes it difficult to isolate and collect the oocytes whereas aspiration method is the most hygienic, practically suitable and preferred method to collect oocytes for IVM/IVF studies.

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REFERENCES