ULTRASONOGRAPHIC BIOMETRY OF THE OVARY AND ITS RESPONSES DURING SUPEROVULATION IN TODA BUFFALOES

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ABSTRACT

Eight superovulated Toda buffaloes were studied ultrasonographically to record the biometry of the ovarian structures and superovulatory response during superovulation and flushing programme, conducted in this breed as a breed conservation measure. Ovarian size (10 buffaloes) and structural changes (eight superovulated buffaloes) were monitored on a) the 10th day post heat (before initiation of FSH) b) Post SOV heat (the 3rd day of superovulatory heat) and c) on the day of flushing. The animals were subjected to superovulation with either 400 or 600 mg FSH (Folltropin V). The superovulation was initiated from the 10th day of the estrous cycle, and embryos were collected on the 5.5 to 6 day of post superovulatory (SOV) heat. Before SOV programming, the average size of the left ovary was found to be 24.67 ± 2.35 mm while the right ovary measured 26.11 ± 1.71 mm and the average size of CL was 14.50 ± 3.28 mm. There was significant increase in the length and width of ovaries post superovulation and on the day of flushing. A greater number of ovarian structures (CL/follicles) were found at the time of flushing than during post SOV heat indicating late/ an-ovulations (post heat). The average size of the follicle showed increase on the day of flushing, due to cystic ovarian condition in a few buffaloes. Late ovulation and a lower number of recruited follicles during superovulation may be the reason for lower response in Toda buffaloes than in other breeds of buffaloes.

Keywords: Toda buffalo, ultrasonography, superovulation, ovary

INTRODUCTION

Buffaloes (Bubalus bubalis) in general are known to be very poor responders to superovulation protocols in comparison to white cattle. The total population of follicles is comparatively lower in buffaloes than in cattle (Madan, 1990). The main problem encountered during superovulation with different hormones based on earlier reports on superovulation was the availability of anovulatory follicle, leading to few and poor quality embryos (Madan et al., 1996 and Misra, 1997). In addition the quality of CL and presence of un-ovulated follicle is also known to influence the recruitment of new follicles.

Several reports suggest a lower follicular population in the buffalo ovaries (Madan, 1990 and Totey et al., 1991). It is essential to know the number of follicles recruited and CL available in buffaloes before and during superovulation and embryo collection programme. Hence an attempt has been made to study ovarian size and structures using ultrasound scanner in the semi wild Toda buffaloes of Nilgiris district of Tamil Nadu, during superovulation. The study was carried out at the Sheep Breeding Research Station, Sandynallah, Nilgiris, in collaboration with Sabarmati Ashram Gaushala, Bidaj Farm, Gujarat, under the conservation project funded by the Department of Biotechnology, Government of India and the National Dairy Development Board.

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MATERIALS AND METHODS

Ten female Toda buffaloes were purchased from Toda hamlets (Toda munds) and were managed under a semi-intensive system of management at the Sheep Breeding Research Station, Sandynallah, The Nilgiris. During the day time, the animals were allowed to graze on natural pastures of the farm land. The animals were fed with 2 kg of concentrate ration per day per animal.

The work was undertaken during December 2006. The estrum in Toda buffaloes were synchronized with two injections of prostaglandin, (inj. Iliren- 5 ml i/m; Hoechst, India) 11 day apart. Post second PG, animals were checked per-rectally at 72 h for the presence of follicle on ovary, uterine tone and discharge. The animals reporting for estrum were selected for superovulation. Eight buffaloes were superovulated using either 400 mg or 600 mg of NIH-FSH-P1 (Folltorpin-V, Vetrepharm, Ontario, Canada). FSH was given from the 10th day of the estrous cycle for 5 days in a tapering dose rate. Luteolysis was induced with prostaglandin injection along with a 7th and 8th FSH dose. All the animals reporting to oestrum (48-72 h post PG) were allowed to be bred by the Toda bull. The superovulated animals were flushed on the 5.5 or 6th day after breeding. The number of corpora lutea was counted per-rectally before flushing. Flushing was carried out as per the standard procedures (Misra et al., 1980) using 18 gauge Rusch catheter (Minitub, Germany) and DPBS media (IMV, France) with 0.1 % Bovine Serum Albumin (Sigma) added.

An ultrasound scanner was used to record the ovarian structures and superovulatory response in these animals during the superovulation and flushing programme. Ovarian size (10 buffaloes) and structural changes (8 superovulated buffaloes) were monitored on a) the 10th day post heat (before initiation of FSH) b) post SOV heat (3rd day) and c) on the day of flushing.

A real time B-mode ultrasound scanner (Medison SA600V, BCF Technology Ltd., Scotland) equipped with a 5.0 MHz linear-array rectal transducer and a video graphic printer (Sony, Japan) was used for this study. The total follicle population was recorded as appreciated by anechoic black structures, while CL with granular structures and more echogenicity were recorded and measured. For comparison, two pairs of ovaries were collected from Toda buffaloes from a slaughter house. The ovarian size and structure were recorded.

The means and standard errors for all variables were calculated and presented. Differences between the ovarian size and the number of follicles and corpus luteum before and after superovulation were tested by Student “t” test.

RESULTS AND DISCUSSION

Buffaloes are regarded to have a lower reproductive efficiency and several reports suggest lower follicular population in the buffalo ovaries (Madan, 1990 and Totey et al., 1991). The mean length, width and height of ovaries in slaughter specimens were 31.00 ± 5.00, 13.50 ± 0.50 and 14.00 ± 0.00 mm (Lt. Ovary) and 27.50 ± 1.50, 25.00 ± 2.00 and 14.50 ± 4.50 mm (Rt. Ovary), respectively (Table 1). The mean length, width and height of ovaries of Toda buffaloes were greater than those observed in non-descript buffaloes by Chandrahasan and Rajasekaran (2004) and in Murrah buffaloes by Kumar et al. (2004). Both left ovaries in slaughter specimens had mature projecting CL of 15 mm and 10 mm in size. One of the right ovaries had a graffian follicle of 15 mm in size.

Significant superovulatory changes in the length and width of ovaries prior to and post superovulation were observed. The smallest normal ovary was found to be 13 x 10 mm (length x width), while the biggest ovary measured 37 x 29 mm. On the day of flushing, they measured 17 x 17 mm and 42 x 36 mm, respectively. The length and width of ovary as observed with ultrasonography in this study was higher than that found by Chandrahasan and Rajasekaran (2004) in non-descript buffaloes and Kumar et al. (2004) in Murrah buffaloes. Use of FSH increased the size of ovary significantly at post SOV heat. Also significant increase (P<0.05) in the size of the ovary on the day of flushing was observed. The changes in ovary size and structures during superovulation are shown in Figure 1.
Corpus luteum was present in all the animals studied on the day of SOV, and there was an increase (1.67 ± 0.24) in the number on the day of flushing (Table 3). Similarly, there was an increase in the availability of number of follicles in response to superovulation. The average size of follicle on the day of flushing (10.25 ± 1.28) was greater as compared to the 10th day (9.00 ± 0.82).

On the 10th day post heat, six buffaloes were shown to have a distinct CL, while in two buffaloes, CL was not found in any of the ovaries. However, both the buffaloes had follicles in their ovaries. There was an increase in number of CL on the day of flushing (1.67 ± 0.24) compared to the number found on the 10th day (1.00 ± 0.00). However, there was no difference in the number of CL on post SOV heat (1.00 ± 0.00) and the 10th day. Similarly, there was no difference in the availability of follicles on post SOV heat (4.00 ± 0.44) and on the day of flushing (3.64 ± 0.61). Both these findings indicate that the buffaloes in this study had late ovulations (post heat), and that the number of recruited follicles even during superovulation was low.

Overall, there was no significant response in the presence of ovarian structures on the 10th day or post SOV heat or on flushing day. The presence of a lower number of primordial follicles and poor recruitment of follicles on the 10th day of cycle may be the reason for the lower response. The current results are in agreement with the findings of Madan (1990), who showed that buffaloes have a low number of primordial follicles at the 10th day of the estrous cycle. However, Chandrahasan and Rajsekaran (2004) found a greater number (3.41 ± 0.11) of follicles than Toda buffaloes (2.80 ± 0.63). Rohilla et al. (2005) also found 7.7 ± 0.3 follicles in anoestrus Murrah buffaloes by ultrasonography.

The size of the follicle observed in this study was comparable to the ultrasonographic studies by Honparkhe et al. (2003) and Rohilla et al. (2005). The average size of the follicle on the flushing day (10.25 ± 1.28 mm) was greater as compared to the 10th day (9.00 ± 0.82 mm). This increase in size of the follicle may be due to the presence of cysts (16-22 mm) found on the day of flushing in three buffaloes. The size of CL was larger than those studied by Honparkhe et al. (2003) and Chandrahasan and Rajsekaran (2004). In conclusion, Toda buffaloes were found to have large-sized ovaries compared to Murrah buffaloes, but their response to superovulation was very poor, which might be due to a lower number of primordial follicles than in other buffaloes. More study with the use of different hormone regimens along with ultrasonography are required to fully exploit the germplasm of these buffaloes. It was observed that ultrasound can be a very good tool for more detailed, reliable and accurate study of ovarian responses to superovulation in buffaloes.
Figure 1. Ovary and structural changes during superovulation.
Table 1. Ovarian biometry of two pairs of ovaries obtained from a slaughter house.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Side</th>
<th>Length in mm</th>
<th>Width in mm</th>
<th>Height in mm</th>
<th>Corpus Luteum (CL)/Follicle (F)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Left</td>
<td>36</td>
<td>14</td>
<td>15</td>
<td>1.0</td>
<td>1CL, 3F</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>29</td>
<td>23</td>
<td>19</td>
<td>1.0</td>
<td>1CL</td>
</tr>
<tr>
<td>2</td>
<td>Left</td>
<td>26</td>
<td>13</td>
<td>13</td>
<td>1.0</td>
<td>1LF, 1SCL</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>26</td>
<td>17</td>
<td>10</td>
<td>0.0</td>
<td>1SCL</td>
</tr>
</tbody>
</table>

Table 2. Mean (± SE) of superovulatory changes in ovarian size (mm).

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Length</th>
<th>Width</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lt. Ovary</td>
<td>Rt. Ovary</td>
</tr>
<tr>
<td>10th day (prior to SOV)</td>
<td>24.67 ± 2.35a</td>
<td>26.11 ± 1.71</td>
</tr>
<tr>
<td>Post SOV Heat</td>
<td>30.63 ± 1.77b</td>
<td>29.50 ± 2.19</td>
</tr>
<tr>
<td>Flushing day</td>
<td>33.71 ± 1.69b</td>
<td>31.00 ± 3.42</td>
</tr>
</tbody>
</table>

Means in the same column within categories with different superscript differ significantly (p<0.05).

Table 3. Mean (± SE) of number of ovarian structures and their size (mm) during superovulation.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Number available</th>
<th>Average Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CL</td>
<td>Follicle</td>
</tr>
<tr>
<td>10th day (prior to SOV)</td>
<td>1.00 ± 0.00</td>
<td>2.80 ± 0.63</td>
</tr>
<tr>
<td>Post SOV Heat</td>
<td>1.00 ± 0.00</td>
<td>4.00 ± 0.44</td>
</tr>
<tr>
<td>Flushing day</td>
<td>1.67 ± 0.24</td>
<td>3.64 ± 0.61</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

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REFERENCES


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