ABSTRACT

The ovarian follicular and luteal developmental patterns of Jersey crossbred cattle (n = 6) and Murrah graded buffaloes (n = 6) were compared to test the hypothesis that the reduced reproductive efficiency in buffaloes when compared with crossbred cattle might be due to variations in follicular and luteal dynamics. The follicular and luteal developmental patterns were studied in two consecutive cycles (n = 12) in all the animals. Ultrasonographic monitoring of ovarian follicular development throughout the oestrus cycle in crossbred cattle revealed that three (25 percent) and nine (75 percent) cycles had two waves and three waves, respectively. In buffaloes, two (16.7 percent) and ten (83.3 percent) cycles had two waves and three waves, respectively. In buffaloes, the dominant follicle (DF) of anovulatory waves reached the maximum size earlier and remained in the static phase for a significantly (P < 0.05) greater number of days (2.0-2.2 days) than in crossbred cows (0.67-1.67 days), which indicated early loss of LH receptors in the dominant follicle (DF) of buffaloes. The mean maximum diameter of the final wave DF (ovulatory) was significantly (P < 0.05) smaller (10.9 ± 0.7 mm) and its growth rate was comparatively slower (1.73 ± 0.10 mm / day) in buffaloes when compared with crossbred cattle (13.33 ± 0.72 mm and 1.95 ± 0.30 mm / day respectively). The smaller diameter and slow growth rate of the ovulatory follicle might be the cause for suboestrus and smaller CL in buffaloes. Thus follicular and luteal dynamics of buffaloes differ significantly in various vital parameters when compared with crossbred cattle, and this might contribute to their lower reproductive efficiency.

Keywords: ultrasonography, follicular waves, corpus luteum, crossbred cows, graded buffaloes

INTRODUCTION

The world buffalo population can be estimated to be roughly 170 million head, of which approximately 97 percent is in Asian countries (Presicce, 2007). Buffaloes are the backbone of the dairy industry in India contributing above 55 percent of total milk production. However, because of their poor breeding capabilities (late maturity, poor oestrus expression, prolonged calving intervals and seasonal reproductive patterns) there has been minimal genetic selection for fertility in buffaloes.
when compared with cattle. In order to optimize buffalo reproduction, the physiological controls of recruitment, selection, growth, dominance and atresia of ovarian follicles need to be better understood. Since manual palpation of genitalia per rectum is not completely accurate in buffaloes because of the anatomical disposition and smaller structures of the ovaries (Saini et al., 2007), the accuracy of real-time ultrasonography is a reliable method for arriving at the follicular and luteal developmental pattern in buffaloes. Extensive ultrasonographic studies have shown clear patterns of follicular dynamics in cattle and thus provided the basis for improving fertility and synchronizing oestrus with more precision in this species. These established reproductive management techniques in cattle can be successfully applied to buffalo because of similarities in the anatomy, physiology and endocrinology of reproduction between the two genera. Even though there have been reports comparing the gross reproductive parameters of buffalo and cattle (Drost, 2007), specific comparative analysis of follicular and luteal development between these two species are lacking. Hence, this study was undertaken to test the hypothesis that the reduced reproductive efficiency in water buffaloes (Bubalus bubalis) might be due to variations in follicular and luteal dynamics when compared with crossbred cattle (Bos taurus x Bos indicus).

MATERIALS AND METHODS

Healthy Jersey crossbred pluriparous cows (n = 6) and Murrah graded pluriparous buffaloes (n = 6), aged 5-6 years, maintained at the Centralised Embryo Biotechnology Unit, Department of Animal Biotechnology, Madras Veterinary College, Madhavaram, Chennai were selected for the study. All the experimental cows and buffaloes were maintained under ideal and identical stall fed conditions throughout the study. They were fed with adequate concentrates, green fodder, and paddy straw and had access to water ad libidum. They were monitored regularly for oestrus symptoms, and cyclicity of the animals was confirmed by frequent gynaeco-clinical examination.

The follicular and luteal developmental pattern was studied ultrasonographically in two consecutive cycles (n = 12) in all the animals. The ovaries of each cow / buffalo were examined ultrasonically every other day throughout an oestrous cycle starting from observed oestrus (Day 0) to the subsequent oestrus (Sianangama and Rajamahendran, 1996) using a real time B-mode ultrasound scanner (SONOVET 600) equipped with 7.5 MHz transrectal transducer. Data collection involved recording length and width of all detectable follicles ≥ 4 mm in each ovary and corpus luteum (CL) with the inbuilt scale provided with the ultrasound instrument. The diameters of each follicle and CL were determined by taking the mean of the length and width of the respective structures (Zeitoun et al., 1996).

Study of follicular dynamics

Each ovary was scanned and imaged in more than one plane to assure that all measurable follicles of ≥ 4 mm in diameter were detected. The follicle, which exceeded the diameter of all other recruited follicles of a wave by ≥ 2mm and reached the maximum diameter, was determined as the dominant follicle (DF). The day of wave emergence was determined as the day the DF was first detected or retrospectively identified at a diameter of 4 mm
(Bo et al., 1993). If the follicle was not detected until it was \( \geq 5 \) mm, a growth rate of 1.5 mm / 24 h was used to retrospectively determine the first examination when the follicle would have been 4.0 mm (Bergefelt et al., 2003). The growth, static and regression phases of DF during various waves were arrived at as described by Savio et al. (1988). A sketch of the ovaries was made recording the location and diameter of the individual identified follicles of \( \geq 4 \) mm. The single data set was used to profile the day-to-day diameters of individual follicles by the identity method, as described by Ginther (1993) and pattern of follicular waves were arrived at.

**Study of luteal dynamics**

After oestrus, ovulation was confirmed when the ovulatory follicle was no longer seen in the subsequent examination and was retrospectively confirmed with the visible recognition of luteal tissue in the same location (Kim and Kim, 2007). As in follicular study, the CL was also mapped during each examination and diameter was arrived at. The developmental pattern of the CL, the day at which it attained the maximum diameter and the regression pattern were recorded (Taponen et al., 2000). Corpus luteum area (CLA) during the mid cycle (Day 10) was calculated using a formula: CL length\( \times 0.5 \)xCL width\( \times 0.5 \times 3.14 \) (Kastelic et al., 1990).

**Statistical analysis**

Data on follicular and luteal characteristics in normal oestrous cycles of cows and buffaloes were analysed by student’s t-test and by analysis of variance (ANOVA) with completely randomised design (Snedecor and Cochran, 1994). SPSS.10.0* software was used for analysis of data.

**RESULTS**

**Number of follicular waves**

Ultrasonographic monitoring of normal follicular wave pattern in crossbred cattle revealed that, nine (75.0 percent) and three (25.0 percent) oestrous cycles had three-and two-follicular wave patterns, respectively. In buffaloes, ten (83.3 percent) and two (16.7 percent) oestrous cycles had three- and two-waves, respectively. The incidence of three-wave cycles was significantly higher (\( P < 0.01 \)) than two-wave cycles in both cattle and buffaloes. Since the incidence of two-wave cycles was statistically insignificant, only three-wave cycles were taken for comparative analysis between crossbred cattle and buffaloes.

The follicular wave pattern and characteristics of DFs of various waves during the oestrous cycle of Jersey crossbred cows and Murrah graded buffaloes are presented in Table 1.

**Mean inter-oestrus interval**

The oestrous cycle length was found to be greater in crossbred cows (22.67 ± 1.20 days) than in buffaloes (21.80 ± 0.54 days), but there was no significant difference between them.

**Follicular wave emergence**

The day of emergence of the first follicular wave was significantly (\( P < 0.05 \)) earlier (Day 0.89 ± 0.31) and the emergence of second wave was significantly (\( P < 0.05 \)) later (Day 9.44 ± 0.34) in crossbred cows when compared with buffaloes (Day 1.80 ± 0.26 and 8.60 ± 0.20 respectively). However, there was no significant difference in the day of emergence of the third wave (Day 15.67 ± 0.29 and 15.40 ± 0.72 respectively) between them.
Characteristics of dominant follicles

Dominant follicles of anovulatory waves

In buffaloes, the DF of the first-wave reached the maximum size significantly (P < 0.01) earlier (Day 5.80 ± 0.50) at a significantly (P < 0.01) faster growth rate (2.10 ± 0.14 mm / day) and remained in the static phase for a significantly (P < 0.05) greater number of days (2.20 ± 0.70) than in crossbred cattle (Day 7.33 ± 0.58, 1.58 ± 0.09 mm / day and 1.67 ± 0.66 days respectively). Similarly, the second-wave DF reached the maximum size non-significantly earlier in buffaloes than in the crossbred cows and remained static for a significantly (P < 0.01) longer duration (2.00 ± 0.41 vs 0.44 ± 0.29 days respectively).

Dominant follicle of ovulatory wave

In the present study, the mean maximum diameter of third-wave DF (ovulatory follicle) was significantly (P < 0.01) smaller in buffaloes (10.9 ±0.7) when compared with the crossbred cattle (13.33 ± 0.72). The growth rate of ovulatory follicles was non-significantly slower in buffaloes.

Characteristics of corpus luteum

In the case of buffaloes, the CL reached the maximum diameter (17.04 ± 0.36 mm) on Day 7.17 ± 0.58, after which the size significantly (P > 0.01) reduced to 12.13 ± 0.26 mm on Day 11.92 ± 0.31, then gradually increased to 15.29 ± 0.38 mm on Day 16.92 ± 0.45 and regressed constantly thereafter. In crossbred cows, the CL grew to a mean maximum diameter of 21.58 ± 0.36 mm on the mean Day of 9.33 ± 0.51 and remained fluctuating around this diameter till Days 14 or 15 of the cycle. Initiation of constant luteal regression occurred from the mean Day of 16.25 ± 0.76.

The CL diameter and the CLA during the mid cycle (Day 10) in buffaloes were 14.42 ± 1.0 mm and 164.60 ± 22.6 mm², respectively. The respective values in crossbred cattle were 20.83 ± 0.41 mm and 330.22 ± 16.0 mm². Crossbred cows had a significantly (P> 0.01) larger CL than buffaloes.

DISCUSSION

Number of follicular waves

In the present study, the incidence of three-wave cycles was significantly higher (P < 0.01) than two-wave cycles in both cattle and buffaloes. Sirois and Fortune (1988) supported the three-wave hypothesis and stated that 80 percent of the oestrous cycles in cattle had more than two follicular waves. However, Singh et al. (1996) observed one, two and three follicular waves in 4.3, 65.2 and 30.5 percent of oestrous cycles, respectively, and reported that the average number of follicular waves in crossbred cows was 2.3 ± 0.1 per cycle. The studies by Taneja et al. (1996) in buffaloes confirmed the development of the ovarian follicles occurring in one or two-waves per oestrous cycle. Subsequently, three-wave oestrous cycles were reported in Murrah buffaloes (Baruselli et al., 1997; Warriach and Ahmad, 2007), but with higher incidence of two-wave cycles. However, in the present study, there was a significantly (P < 0.01) higher incidence of three-wave cycles than two-wave cycles in concurrence with the observation of Barkawi et al. (2009) in buffaloes. The variations in number of follicular waves were attributed to many factors like, status of nutrition, lactation, environment etc. at the time of study (Lucy et al., 1992). The days of wave emergence in cattle and buffaloes were similar to the reports of Ginther et al. (1989); Baruselli et al. (1997), respectively. Even though the oestrous cycle length
was found to be greater in crossbred cows (22.67 ± 1.20 days) than in buffaloes (21.80 ± 0.54 days), there was no significant difference between them. The findings coincided with the earlier reports of Malhi et al. (2005) in cattle and Drost (2007) in water buffaloes.

**Characteristics of dominant follicles**

It was reported that the first wave DFs have more consistent characteristics than the subsequent waves (Ginther et al., 2003) providing ample flexibility for regularizing the oestrous cycle. On perusal of the data in the present study, it was obvious that the characteristics of DF of first follicular wave were significantly varying in several parameters between buffaloes and crossbred cattle.

In buffaloes, the DF of anovulatory waves, i.e., the first and the second waves reached the maximum size earlier and remained in the static phase for significantly greater number of days than in the crossbred cows. Rastegarnia et al. (2004) stated that there will be a decrease in the number of LH receptors in the follicles at the static and regression phases and attributed this feature for lower ovulatory response for gonadotrophin releasing hormone (GnRH) treatment. Thus it was evident that in buffaloes the DFs were losing their LH receptors earlier than crossbred cattle, and in turn, their capacity to respond to endogenous or exogenous luteinizing hormone. These factors should be taken into account when designing protocols for oestrous / and follicular wave synchronization studies in buffaloes.

The third-wave DF (ovulatory follicle) in buffaloes developed at a slow pace and reached a maximum diameter (10.9 ±0.7mm) which was significantly smaller than its counterpart in crossbred cattle (13.33 ± 0.72mm). The individual observations were in accordance with the reports of Taneja et al. (1996); Drost (2007). Suboestrus or silent oestrus constitutes the single largest problem affecting the reproductive efficiency in water buffaloes thereby increasing the inter-calving period. Awasthi et al. (2007) correlated the slower growth rate and smaller size of the ovulatory follicle with silent oestrus condition in buffaloes and suggested that the DF in animals with silent oestrus grew slowly due to lower availability of LH to developing follicle which affected the bioavailability or synthesis of various growth factors and/or their binding proteins needed for terminal growth of the follicle. As the expression of oestrus behavior was dependent on amount of oestrogen produced by granulosa cells, it might be attributed to impaired oestradiol production which in turn was dependant on androgen production from theca cells under the influence of LH. However, further research is needed to identify the factor(s) affecting the growth rate of the ovulatory follicle resulting in silent oestrus in buffaloes.

**Characteristics of corpus luteum**

Overall observation during an oestrous cycle revealed that the crossbred cows had a significantly (P > 0.01) larger CL than buffaloes. Sartori et al. (2002) reported a significant positive correlation between size of the ovulatory follicle and size of the CL. It seems likely that increased ovulatory follicular size would lead to increased numbers of granulosa cells which differentiate into large luteal cells following the LH surge and subsequent increased size of the CL (Smith et al., 1994). So the variation in size of the CL between these two species could be at least partially the result of the variation in the size of ovulatory follicles of previous cycles.

In crossbred cows, the CL grew to a peak diameter of 21.58 ± 0.36 mm on the mean Day of
Table 1. Characteristics of dominant follicles of three-wave oestrous cycles in Murrah graded buffaloes and Jersey crossbred cattle.

<table>
<thead>
<tr>
<th>WAVE NO.</th>
<th>Characteristics of dominant follicle</th>
<th>Three-wave oestrous cycles</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Buffaloes (n = 10)</td>
<td>Cows (n = 9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>FIRST</td>
<td>Day of wave Emergence</td>
<td>1.80 ± 0.26</td>
<td>0.89 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>Day of maximum diameter</td>
<td>5.80 ± 0.50</td>
<td>7.33 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>Maximum diameter (mm)</td>
<td>10.0 ± 0.30</td>
<td>11.22 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>Growth Phase (days)</td>
<td>5.00 ± 0.40</td>
<td>7.33 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>Growth Rate (mm/day)</td>
<td>2.10 ± 0.14</td>
<td>1.58 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Static Phase (days)</td>
<td>2.20 ± 0.70</td>
<td>1.67 ± 0.66</td>
</tr>
<tr>
<td></td>
<td>Regression Phase(days)</td>
<td>7.00 ± 0.70</td>
<td>8.0 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>Regression Rate (mm/day)</td>
<td>1.50 ± 0.23</td>
<td>1.47 ± 0.14</td>
</tr>
<tr>
<td>SECOND</td>
<td>Day of wave Emergence</td>
<td>8.60 ± 0.20</td>
<td>9.44 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>Day of maximum diameter</td>
<td>14.00 ± 0.62</td>
<td>15.78 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>Maximum diameter (mm)</td>
<td>10.40 ± 0.70</td>
<td>10.11 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>Growth Phase (days)</td>
<td>7.20 ± 0.70</td>
<td>7.44 ± 0.50</td>
</tr>
<tr>
<td></td>
<td>Growth Rate (mm/day)</td>
<td>1.50 ± 0.10</td>
<td>1.42 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>Static Phase (days)</td>
<td>2.00 ± 0.41</td>
<td>0.44 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>Regression Phase(days)</td>
<td>5.40 ± 0.52</td>
<td>4.89 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>Regression Rate (mm/day)</td>
<td>1.96 ± 0.10</td>
<td>2.24 ± 0.25</td>
</tr>
<tr>
<td>THIRD</td>
<td>Day of wave Emergence</td>
<td>15.40 ± 0.72</td>
<td>15.67 ± 0.29</td>
</tr>
<tr>
<td>(ovulatory)</td>
<td>Day of maximum diameter</td>
<td>21.80 ± 0.54</td>
<td>22.67 ± 1.20</td>
</tr>
<tr>
<td></td>
<td>Maximum diameter (mm)</td>
<td>10.90 ± 0.71</td>
<td>13.33 ± 0.72</td>
</tr>
<tr>
<td></td>
<td>Growth Phase (days)</td>
<td>6.20 ± 0.40</td>
<td>6.83 ± 1.86</td>
</tr>
<tr>
<td></td>
<td>Growth Rate (mm/day)</td>
<td>1.73 ± 0.10</td>
<td>1.95 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>Oestrous cycle length</td>
<td>21.80 ± 0.54</td>
<td>22.67 ± 1.20</td>
</tr>
</tbody>
</table>

** (P < 0.01)    * (P < 0.05)    NS-Not significant (P > 0.05)
9.33 ± 0.51. Taponen et al. (2000) also recorded a similar mean maximum diameter of 22.7± 1.1 mm in Finnish Ayrshire breed cows and heifers, but the size was attained at a later stage of cycle (Days 11 or 12). Similar to our study they also observed a fluctuation around this diameter till Days 14 or 15 of the cycle. Initiation of luteal regression occurred from the mean Day of 16.25 ± 0.76 which corroborated with the observations of Sianangama and Rajamahendran (1996). In the case of buffaloes, the CL reached the maximum diameter (17.04 ± 0.36 mm) on Day 7.17 ± 0.58 in concurrence with the findings of Barkawi et al. (2009). Unlike in cattle, the CL size reduced significantly during the mid luteal phase and regained its growth attaining a second peak before entering the phase of constant regression. Thus there was a significant difference in the luteal developmental pattern between these two species. The CL diameter and the CLA during the mid cycle (Day 10) in buffaloes were 14.42 ± 1.0 mm and 164.60 ± 22.6 mm², respectively. The respective values in crossbred cattle were 20.83 ± 0.41mm and 330.22 ± 16.0 mm². Veronesi et al. (2002) stated that the degree of agreement between plasma progesterone concentrations and diameter of CL was highly significant. Thus the smaller CL would have contributed for low progesterone secretion thereof during the mid luteal phase, a period which was critical for embryonic sustenance, in buffaloes when compared with crossbred cows.

From the foregoing observations, it was concluded that ovarian follicular dynamics of Murrah graded buffaloes differed from crossbred cattle with the significantly increased static phase of DFs of anovulatory waves, the smaller size and the slower growth rate of ovulatory follicle. The resultant smaller corpus luteum too experienced a drastic fluctuation in developmental pattern during the mid luteal phase. These cumulative factors could be contributing for the lowered reproductive efficiency in buffaloes.

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