Effect of preovulatory follicle on fertility in Graded Murrah buffaloes (Bubalus bubalis)


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ABSTRACT

An investigation was taken up to study the relationship of preovulatory follicle size at the time of first postpartum AI and CL biometry with conception using transrectal ultrasonography in 70 parous Graded Murrah buffaloes maintained under rural conditions. POF sizes were measured ultrasonographically and grouped in to small preovulatory follicle (SPF), medium preovulatory follicle (MPF) and large preovulatory follicle (LPF). Serum progesterone concentration at the time of AI and pregnancy status was negatively correlated indicating that when progesterone level declined to < 0.3 ng/ml (basal level) at the time of AI, the chances of the animal becoming pregnant was increased. The size of the POF was positively correlated to the size of the CL on day 10 in pregnant buffaloes. The overall POF diameter at the time of estrum was 12.31 ± 0.29 mm (Range 9 to 16 mm) in Graded Murrah buffaloes and 50% of the buffaloes had POF size > 12 - ≤14 mm. They conceived only if the POF diameter was more than 9 mm at the time of AI however there was no significant correlation between the POF size at the time of AI and conception. Thus, it was concluded that physiological maturity rather than the diameter of the follicle influenced the fertility in Graded Murrah buffaloes under field conditions.

Key words: Graded murrah buffalo, Preovulatory follicle, Transrectal ultrasonography.

INTRODUCTION

Buffalo population in the world is continuously increasing and was estimated to be 199 million (FAOSTAT, 2012) with more than 96% of the population located in Asia. Although, it played an important role in all agricultural revolutions, green, white and red, unfortunately it has been neglected owing to its poor breeding ability. This situation warrants some corrective measures for accurate, precise estrus detection by monitoring the ovarian structures like pre-ovulatory follicle and corpus luteum through transrectal ultrasonography (Pandey et al., 2011 and Rahman et al., 2012). Some studies are indicating a positive correlation between the diameter of POF and plasma estradiol concentration and subsequent pregnancy (Noseir, 2003 and Perry et al., 2007). Review of literature revealed paucity of critically derived information on follicular and luteal structures in relation to pregnancy in buffaloes maintained under rural conditions, indicating that no systematic study has been undertaken in these areas. Further, there was no information regarding the percentage of buffaloes reported for AI by the owners at right time so as to achieve optimum fertility rate. Hence, the present study was undertaken to access the effect of POF size, ovulation, CL biometry and serum progesterone at the time of AI with conception in Graded Murrah buffaloes under rural conditions.

MATERIALS AND METHODS

Selection of animals: The present study was conducted at TVCC, NTR CVSc, Gannavaram from the month of Oct, 2014 to May, 2015. A total of 70 buffaloes maintained under village system of rearing free from apparent pathological abnormalities of the reproductive tract. Visual examination of the animal for external signs of estrus and per rectal palpation of genitalia for recording open cervix and tonic uterine horns with expulsion of mucus discharge upon milking of tubular genitalia and presence of palpable follicle on either of the ovaries. They were considered to be in estrus when the CL was not detected and with presence of largest follicle with slight fluctuation to touch (12-15 mm), the uterus showed very strong contractility and the external os of the cervix was open (Lopez-Gatius et al., 1991). Once the estrus was confirmed, the estrus intensity was studied. Based on the estrus symptoms, intensity of estrus was classified as weak, intermediate and intense, on the basis of score card devised by Rao and Rao (1981) with slight modification.

Transrectal Ultrasonography: Transrectal ultrasonographic monitoring (Real time B mode) of ovarian follicles, CL and pregnancy was carried out with 7.5 MHz linear array rectal
transducer (prosound, aloka, japan) The scanned images were frozen and size of the follicle/CL was determined by the measurement of the largest and smallest diameter of the follicle/CL and thereafter average diameter was calculated using inbuilt scale provided within the ultrasound scanner. Preovulatory follicle size at the time of AI (on day 0 at estrus) are grouped as small preovulatory follicle (SPF <9 to ≤12 mm), medium preovulatory follicle (MPF >12 to ≤14 mm) and large preovulatory follicle (LPF >14 mm) (Rahman et al., 2012). Double AI was performed on the day of estrus with frozen thawed semen (French mini straws 0.25 ml, 30 million spermatozoa per straw) after checking the semen for its post thaw motility (50-60 per cent) from a proven sire supplied by APLDA. Ovulation was confirmed by the disappearance of the preovulatory follicle at the same site with subsequent formation of luteal tissue on the next day of examination monitored by ultrasound. Persistence of the preovulatory follicles leading to prolonged duration of heat for a period of 2-3 days was considered as delayed ovulation. The animals are re-examined using ultrasound on day 10 following insemination for the presence of CL which was measured in a similar manner as the follicles. All the buffaloes were examined transrectally for pregnancy diagnosis at day 30 post AI which was confirmed on per rectal examination on day 60.

Blood sampling and RIA: Blood samples were obtained from 40 buffalo cows at the time of AI and on day 10 via venipuncture (jugular vein) into 5 ml vacuum tubes to determine serum progesterone concentration. Blood was allowed to coagulate at room temperature, stored at 4° C for 24 h, and centrifuged at 1200 RPM for 30 min. Serum was harvested and stored at - 20°C until Radioimmunoassay (RIA). Serum progesterone was determined using a commercial solid-phase RIA kit containing anti progesterone antibody-coated tubes, and 1\(^{125}\)I-labeled progesterone (Immunotech, Beckman Coulter, France) (Pfeifer et al., 2009). The serum progesterone concentration was expressed in nanograms per milliliter (ng/ml). All statistical analysis was performed using the SPSS (20.0) system for Windows.

RESULTS AND DISCUSSION

Out of 70 buffaloes scanned for POF size, 15, 35 and 20 buffaloes recorded as SPF (<9 to ≤12 mm), MPF (>12 to ≤14 mm) and LPF (>14 mm), with group means of 10.53±0.19, 13.36±0.09 and 15.4±0.13 mm, respectively, with a mean diameter of 12.31±0.29 ranging from 9 to 16 mm. These findings are almost concurring, with the findings of Baruselli (1997) in Murrah buffaloes (13.3±1.8 mm) and Presicce et al. (2005) in primiparous and pluriparous Mediterranean Italian buffaloes during postpartum estrus with a mean POF size of 13.5±0.8 mm. Similar findings were also reported, in Iraqi buffaloes by Azawi et al. (2009), who reported the POF size, on the right and left ovaries as 10.78 and 11.24±2.15 mm, respectively. Barkawi et al. (2009) reported the maximum diameter of POF as 14-15 mm in Egyptian buffaloes whereas Yindee et al. (2011) in swamp buffaloes revealed the presence of ovulatory follicles with a mean diameter of 13.5±0.52 to 14.17±1.08 mm during the post-partum ovulations. Contrary to the present findings, Derar et al. (2012) reported the POF size as 9.8±0.32 mm in Egyptian buffaloes which was much lower than the present observations. However, Neglia et al. (2007) reported the ovulatory follicle size as 15.3±0.03 mm in non-lactating Murrah buffaloes and in Italian Mediterranean buffaloes (16.9±0.16 vs 14.9±0.25 mm), respectively which was greater than the diameter of pre ovulatory follicle size observed in the present study. These differences could be due to location, season, feeding, managerial conditions. The overall mean diameter of the POF in pregnant group (12.71±0.39) was slightly larger than nonpregnant group (11.91±0.42) buffaloes but the difference was not statistically significant (P>0.05) (Table. 1). The POF diameter is negatively correlated with progesterone concentration on day 10 which is statistically highly significant (Table. 2). The ultrasonic determination of diameter of CL on day 10 post AI was carried out and grouped as >12 to ≤15, >15 to ≤18 and >18 mm with mean values of 13.77±0.23, 17.06±0.21 and 20.16±0.40 mm, respectively. The overall mean CL size in the present trial was 15.60±0.49 mm having a range of

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Overall</th>
<th>Pregnant</th>
<th>Non pregnant</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrus intensity (Score) (Range)(n)</td>
<td>1.93±0.06</td>
<td>2.06±0.09</td>
<td>1.80±0.08</td>
<td>0.234</td>
</tr>
<tr>
<td>Preovulatory follicle size (mm)(Range)(n)</td>
<td>12.31±0.29</td>
<td>2.71±0.39</td>
<td>11.91±0.42</td>
<td>0.174</td>
</tr>
<tr>
<td>Serum Progesterone concentration on estrus day (0) (ng/ml)(Range)(n)</td>
<td>0.67 ± 0.12</td>
<td>0.89±0.13</td>
<td>1.42±0.23</td>
<td>0.065</td>
</tr>
<tr>
<td>Corpus luteum size (mm) (Range)(n)</td>
<td>15.60± 0.49</td>
<td>7.83±0.31</td>
<td>14.90±0.92</td>
<td>0.004</td>
</tr>
<tr>
<td>P4 Serum Progesterone concentration on day 10 (ng/ml)(Range)(n)</td>
<td>3.04± 0.06</td>
<td>3.09±0.07</td>
<td>2.86±0.10</td>
<td>0.041</td>
</tr>
</tbody>
</table>

** Highly significant (P<0.01), * Significant (P<0.05), NS-Non significant (P>0.05)
Table 2: Correlation between different estrus parameters in graded murrah buffaloes maintained under village conditions.

<table>
<thead>
<tr>
<th>Correlation between variables</th>
<th>r-value</th>
<th>P value</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy status x Intensity of estrus</td>
<td>-0.752</td>
<td>0.247</td>
<td>NS</td>
</tr>
<tr>
<td>Pregnancy status x POF size</td>
<td>0.906</td>
<td>0.093</td>
<td>NS</td>
</tr>
<tr>
<td>Pregnancy status x Progesterone concentration (on the day of estrus)</td>
<td>-0.933</td>
<td>0.044</td>
<td>*</td>
</tr>
<tr>
<td>POF size x Intensity of estrus</td>
<td>0.746</td>
<td>0.000</td>
<td>**</td>
</tr>
<tr>
<td>POF size x Progesterone concentration (on the day of estrus)</td>
<td>-0.534</td>
<td>0.000</td>
<td>**</td>
</tr>
<tr>
<td>POF size x CL size on day 10</td>
<td>0.834</td>
<td>0.000</td>
<td>**</td>
</tr>
<tr>
<td>Progesterone concentration x Intensity of estrus</td>
<td>-0.536</td>
<td>0.89</td>
<td>NS</td>
</tr>
</tbody>
</table>

** Highly significant (p< 0.01), * Significant (p<0.05), NS-Non significant (P>0.05)

10-21 mm with overall mean progesterone concentration on day 10 was 3.04±0.06 ng/ml. The difference in CL size between pregnant and nonpregnant buffaloes was significant (17.83 vs 14.9 mm, P<0.05) (Table 1). The size of CL was significantly different (P<0.05) among the three groups of POF. The CL size in SPF, MPF and LPF was 14.09±0.61, 16.00±0.48 and 18.90±0.60 mm, respectively. The diameter of POF at AI positively correlated with the diameter of CL at day 10 after AI which is statistically highly significant (r = 0.857, P<0.01). The progesterone concentrations to the different sizes of CL were 2.92±0.08, 3.03±0.70 and 3.39±0.10 ng/ml, respectively with significant difference which suggests that the size of the CL was influenced by the size of POF at the time of estrus (Table 2). The ovulation rate was significantly lower in buffaloes that had POF size 9 to ≤12 mm (LPF) compared to other two groups whereas pregnancy rate was highest (51.43 per cent) in MPF group. The maximum diameter of the POF at the time of AI was positively correlated with the conception rate but it is statistically nonsignificant (r = 0.906, P>0.05) (Table 2). The mean CL size was 15.60±0.49 mm (range 10-21 mm) on day 10 post AI of this study is lesser than the CL size of 18.90 ± 1.20 mm as observed in Egyptian buffaloes (Derar et al. 2012). Similar findings were also reported by Alejandro et al. (2014) with a luteal diameter of 19.58±4.16 mm and 17.74±3.32 mm for pluripara and nullipara buffaloes respectively. Barile et al. (2007) did not find a steady correlation between the size of a POF and that of the subsequent CL. According to George et al. (2005) and ovolution of small follicles (11.5±0.2 mm) results in the development of small CLs and secrets less progesterone than that in cattle induced to ovulate larger follicles (14.47±0.39 mm). In the present study, the mean progestrone level on day 10 at the time of presence of a mean CL of 15.60±0.49 mm was found to be 3.04±0.06 ng/ml. Serum progesterone on the day of estrus was analyzed in 40 buffaloes and with a mean value of 0.67±0.12 ng/ml. The data were classified into three categories namely, buffaloes that had basal level of progesterone <0.3 ng/ml between 0.35 to 1 ng/ml and >1ng/ml at the time of first AI with mean values of 0.23±0.10, 0.89±0.05 and 1.20±0.17 ng/ml, respectively. The ovulation rate for the buffaloes that had < 0.3ng/ml and >0.3 to 1 ng/ml was 100 per cent and 90.90 per cent respectively, indicating that all the buffaloes that had<1ng/ml of progesterone at the time of first AI have ovulated. The ovulation rate was significantly lower (P<0.01) in buffaloes that had serum progesterone value of > 1ng/ml compared to buffaloes that had serum progesterone concentration of < 0.3ng/ml. The pregnancy rate was higher (65.90 per cent) in buffaloes that had < 0.3ng/ml progesterone concentration compared to buffaloes that had progesterone concentration between 0.3–1ng/ml (38.88 per cent) and >1 ng/ml (0 per cent) at the time of AI. The mean values of serum progesterone in pregnant and nonpregnant buffaloes were 0.89±0.13 and 1.42±0.23 ng/ml, respectively which was not statistically significant (P>0.05) (Table.1).The overall ovulation rate was 94.28 per cent which concurs the earlier reports on ovulation rate of 90-93 per cent which was recorded in cyclic buffaloes (Paul and Prakash, 2005). The maximum POF diameter was positively correlated with the estrus intensity and is highly significant (P<0.01). The ovulation rates were higher in buffaloes that exhibited intermediate estrus whereas pregnancy rates were higher in intense estrus group however statistical analysis of the data failed to establish any significant difference (P>0.05) between these groups. The overall conception rate was 50 per cent which was lower than the 63.63 per cent conception recorded by Verma et al. (2014) with accurate estrus detection as observed in the present study, but in the same study Verma et al. (2014) in control group without concern to estrus detection the conception was 41.93 per cent. The conception of this study was, much higher than the earlier reports of Thirunavukarsu and Kathiravan (2009) who reported 25.52 per cent in buffaloes in India and Anzar et al. (2003) 29 per cent in Punjab, Pakistan under field conditions. In the present study, the mean progesterone level at the time of AI or estrus (Day 0) was found to be 0.67±0.12 ng/ml which is in close agreement with several earlier studies which reported the blood progesterone concentration, in blood plasma of Murrah buffaloes at estrus with 0.1ng/ml which rose to a peak of 3.6ng/ml on day 13. It continued to increase in animals that conceived but dropped to 0.6ng/ml on 3 days before the next estrus in those that failed to conceive as reported by (Arora and Pandey, 1982) with basal levels (0.1-0.3ng/ml) during estrus and remains close to 1ng/ml for the next 3-4days. Takkar et al. (1982) reported the
progesterone levels as 0.360±0.062 and 0.334 ± 0.066 ng/ml on the day of estrus in buffaloe heifers and buffaloe cows, respectively. The values were around 1ng/ml till day 6 followed by a gradual increase to a peak average value of 4.888±0.399 and 5.119±0.415 ng/ml on day 15 of the cycle in heifers and cows respectively. Mondal et al. (2010) reported the plasma progesterone concentration as 0.30±0.06 to 1.94±0.03 ng/ml during the estrus cycle in buffaloes. Plasma levels which were lowest during peri estrus phase increased to 0.47±0.70 ng/ml during early luteal phase and then further to 1.94±0.30 ng/ml during the mid-luteal phase. Peak progesterone values of 4–5.1 ng/ml have been recorded about 15 days after estrus (Arora and Pandey, 1982 and Takkar et al., 1982). In the present investigation, the progesterone concentration at the time of AI or estrus (day 0) was significantly different (P<0.01) among the three groups of POF Viz., SPF, MPF and LPF with 0.92±0.20, 0.76±0.17 and 0.40±0.14 ng/ml, respectively. Inadequate luteolysis prior to estrus could have resulted in higher circulating progesterone level near AI and subsequently resulting in anovulation (Wiltbank et al., 2002). In this study, more number of buffaloes became pregnant, when the progesterone concentrations at the time of AI were below suprabasal level and the pregnancy reduced when the progesterone level is between suprabasal and 1 ng/ml. None of the cows become pregnant when the progesterone level was more than 1 ng/ml. The present result concurs with the findings of De Silva et al. (1981) who reported that higher progesterone level at the time of estrus might affected sperm and ovum transport, as well as the fertilization process and subsequent embryo passage to the uterus. The present result is also in agreement with Duchens et al. (1995) reported that supra-basal progesterone level will delay the ovulation and lead to retention of graafian follicle for an extended period and cause damage of the oocyte to such an extent that even inseminating close to the time of ovulation may not ensure fertilization. Thus it was concluded that physiological maturity rather than the diameter of the follicle influenced the fertility, in Graded Murrah buffaloes under field conditions.

REFERENCES


FAO (2012) FAOSTAT agriculture data. Production data.


