



## Relationship of Body Condition Score at Estrus and Conception Rate in Graded Murrah Buffaloes

M. Praveen Raj<sup>1\*</sup>, G. Venkata Naidu<sup>2</sup>, M. Srinivas<sup>3</sup>, M. Raghunath<sup>4</sup> and K. Ananda Rao<sup>5</sup>

<sup>1</sup>Department of Veterinary Gynaecology & Obstetrics, College of Veterinary Science, Tirupati, Andhra Pradesh, INDIA

<sup>2</sup>Department of Veterinary Gynaecology & Obstetrics, NTR CVSc, Gannavaram, Andhra Pradesh, INDIA

<sup>3</sup>Department of Veterinary Gynaecology & Obstetrics, NTR CVSc, Gannavaram, Andhra Pradesh, INDIA

<sup>4</sup>Teaching Veterinary Clinical Complex, NTR CVSc, Gannavaram, Andhra Pradesh, INDIA

<sup>5</sup>Senior Scientist, Buffalo Research Station, Venkataramanna Gudem, Andhra Pradesh, INDIA

Corresponding author: MP Raj; Email: praveenraj0832@gmail.com

Received: 06 May, 2016

Accepted: 28 July, 2016

### ABSTRACT

Seventy graded Murrah parous buffaloes presented for the first postpartum AI maintained under village system of rearing free from apparent pathological abnormalities of the reproductive tract were selected to study the effect of body condition score (BCS) and size of the preovulatory follicle on conception rate. After evaluating Body condition score (BCS) by visual examination and estrus intensity based on the score card, Preovulatory follicle (POF) sizes were measured ultrasonographically and grouped as small preovulatory follicle (SPOF), medium preovulatory follicle (MPOF) and large preovulatory follicle (LPOF). The mean values of BCS, intensity of estrus and serum progesterone at AI and on day 10 post AI were estimated and the relation to conception rate was analyzed. Positive correlation was observed with the body condition of the buffaloes at the time of AI to POF size and intensity of estrus. The mean values of BCS in pregnant and non pregnant buffaloes did not differ significantly and it has a nonsignificant ( $P>0.05$ ) negative correlation with pregnancy status, however proper nutrition during breeding season is necessary for acceptable reproduction. It was concluded from the present study that physiological maturity of the follicle rather than its diameter influenced the fertility in graded Murrah buffaloes under field conditions.

**Keywords:** Body condition score, preovulatory follicle, murrah buffalo

The BCS system is a subjective scoring method for evaluating the energy reserves of dairy animals which provides a better understanding of reproductive parameters. It is based on evaluation of outer appearance of the animal that interacts with its body fat reserves and therefore directly influenced by energy balance. It gives an immediate appraisal of the body state of the animal and is readily incorporated in operational decision-making (Gransworthy, 1988). It is a universally accepted, non-invasive, quick and inexpensive method to estimate the degree of fatness. It aids in the management of the body fat reserves for better health, production and reproduction. Perusal of literature revealed, scanty reports with regards to BCS in relation to preovulatory follicle size and conception rate in graded Murrah buffaloes. Hence the

present study was undertaken to study the effect of BCS on POF size at the time of AI and conception.

### MATERIALS AND METHODS

The present study was conducted at Teaching Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram from the month of October, 2014 to May, 2015. A total of 70 parous buffaloes maintained under village system of rearing free from apparent pathological abnormalities of the reproductive tract were selected and used for this study. BCS was determined by visual examination in all the experimental buffaloes on a scale of 1 (thin) to 5 (fat) with 0.25 unit increase based on evaluation of body areas as per the procedure described by Alapati *et al.* (2010).

Estrus detection was based on visual examination of the animal for external signs of estrus and per rectal palpation of genitalia for an open os of the cervix and tonic uterine horns with mucus discharge upon massage of tubular genitalia and presence of palpable follicle on either of the ovaries. Buffaloes were considered to be in estrus when the CL was not detected and with the presence of a large follicle with slight fluctuation to touch (12-15 mm), uterus showed tonocytivity with 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 scored, based on the estrus symptoms and the 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 ultrasonographic monitoring (Real time B mode) of ovarian follicles was carried out with 7.5 MHz linear array rectal transducer (PROSOUND, ALOKA, JAPAN). The optimal 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 were grouped as small preovulatory follicle (SPOF 9 to 12 mm), medium preovulatory follicle (MPOF >12 to 14 mm) and large preovulatory follicle (LPOF >14 mm) (Rahman *et al.*, 2012). Double inseminations were performed on the day of estrus with frozen thawed semen (French mini straws 0.25 ml, 30 million sperms per straw) from a proven sire supplied by Andhra Pradesh Livestock Development Agency. Ovulation was confirmed by the disappearance of the POF at the same site previously destined site with subsequent formation of luteal tissue on the next day of examination monitored by ultrasound. The animals were re-examined using ultrasound on day 10 post AI for the presence of CL which was measured in a similar manner as that of the follicles and were grouped as >12 to 15, >15 to 18 and >18mm, respectively. All the buffaloes were examined transrectally by using B mode real time ultrasonography for pregnancy diagnosis at day 30 after breeding which was confirmed on per rectal examination on day 60.

Blood samples were obtained from buffaloes at the time of AI and on day 10 via venipuncture (jugular vein) into 5 ml vacutainer 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 I125-labeled progesterone (Immunotech, Beckman Coulter, France). Estimation of progesterone in serum samples was done at the National Institute of Animal Nutrition and Physiology (NIANP), Bengalure (A unit of Indian Council of Agricultural Research) using Packard Gamma Multiwell counters (USA). The sensitivity of the assay was 0.01 ng/ml with intra and inter assay coefficients of variation as 5.8 per cent (n = 10) and 9.0 per cent (n = 18), respectively. The intra-assay and inter-assay variation was assessed as the coefficient of variation of 10 replicate determinations in the same assay and 18 duplicate determinations in different assays, respectively. The serum progesterone concentration was expressed in nanograms per milliliter (ng/ml). Statistical analyses were performed using the SPSS (20.0) for windows.

## RESULTS AND DISCUSSION

The results were tabulated and analyzed. Out of the 70 buffaloes studied, 6, 29, 28 and 7 buffaloes had a BCS 1-2, 2-3, 3-4 and 4-5, respectively. The mean BCS observed at the time of AI was  $2.7 \pm 0.08$  while the mean values in all the individual groups ranged between 2 - 4. The mean values of BCS in pregnant and nonpregnant buffaloes were recorded as  $3.41 \pm 0.12$  and  $2.95 \pm 0.10$ , respectively with no significant difference. The buffaloes with BCS of 1-2, 2-3, 3-4 and 4-5 had a mean POF size of  $10.50 \pm 0.88$ ,  $12.10 \pm 0.42$ ,  $12.29 \pm 0.45$  and  $13.15 \pm 1.35$  mm, respectively with a significant difference ( $P < 0.05$ ) in POF size among the different BCS groups. It was opined that POF diameter was found to be positively correlated with the BCS (Lucy *et al.*, 1991 and Centurion-Castro *et al.*, 2013) and weight of the cattle (Rhodes *et al.*, 1995). Negative energy balance had influenced follicular growth (Beam and Butler, 1997 and Wiltbank *et al.*, 2002) and the size of the ovulated follicle (Armstrong *et al.*, 2001). The mean progesterone concentration on day 0 was  $0.92 \pm 0.53$ ,  $0.73 \pm 0.18$ ,  $0.34 \pm 0.16$  and  $0.19 \pm 0.08$  ng/ml in 1-2, >2-3, >3-4 and >4-5 BCS groups, respectively with a significant difference ( $P < 0.05$ ) among the groups. The overall mean progesterone concentration at estrus was  $0.67 \pm 0.12$  ng/

ml. The conception rate in buffaloes grouped on BCS as 1-2, 2-3, 3-4, 4-5 was 33.33, 63.33, 40 and 50 per cent, respectively and had a nonsignificant ( $P>0.05$ ) negative correlation with pregnancy status (Table 1). Out of 70 buffaloes scanned for POF size, 15(21%), 35(50%) and 20 (29%) buffaloes recorded as SPOF (<9 to 12 mm), MPOF (>12 to 14mm) and LPOF (>14 mm), respectively. The overall mean diameter of the POF in pregnant group ( $12.71\pm 0.39$ ) was slightly larger than nonpregnant group ( $11.91\pm 0.42$ ) buffaloes but the difference was not statistically significant ( $P>0.05$ ). The progesterone concentration on day 0 was significantly different among the three groups of POF viz SPOF, MPOF and LPOF with  $2.92\pm 0.08$ ,  $3.03\pm 0.70$  and  $3.39\pm 0.10$  ng/ml, respectively. The maximum POF diameter was positively correlated with the estrus intensity and BCS which are said to be significant ( $P< 0.01$ ), whereas the POF diameter is negatively correlated with progesterone concentration on day 0 and statistically highly significant ( $P<0.01$ ) (Table 1).

**Table 1:** Correlation between different estrus parameters in graded Murrah buffaloes maintained under village conditions

Correlation between variables	r-value	P value	significance
Pregnancy status $\times$ BCS	-0.645	0.354	NS
Pregnancy status $\times$ POF size	0.906	0.093	NS
POF size $\times$ BCS	0.233	0.026	*
BCS $\times$ Intensity of estrus	0.241	0.022	*

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

This is in accordance with Centurion-Castro *et al.*, 2013 who reported the mean of the diameter of the largest follicles was higher in the BCS5-6 group (12.9 mm) as compared to the follicles for the cows with BCS1-2 (9.8 mm) due to proper growth and maturation of ovarian follicles in turn leading to more estrus intensity supporting the present study. 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  $13.77\pm 0.23$ ,  $17.06\pm 0.21$  and  $20.16\pm 0.40$  mm, respectively. The overall mean CL size was  $15.60\pm 0.49$  mm having a range of 10-21 mm. The overall mean progesterone concentration on day 10 was  $3.04\pm 0.06$  ng/ml. The difference in CL size between pregnant and nonpregnant buffaloes was significant (17.83 vs 14.9 mm) ( $P<0.05$ ). The CL size in SPF, MPF

and LPF was  $14.09\pm 0.61$ ,  $16.00\pm 0.48$  and  $18.90\pm 0.60$  mm, respectively. The progesterone concentrations to the different sizes of CL were  $2.92\pm 0.08$ ,  $3.03\pm 0.70$  and  $3.39\pm 0.10$  ng/ml, respectively with a significant difference ( $P< 0.05$ ). The results suggest that the size of the CL was influenced by the size of POF at the time of estrus (Table). Similar findings were also reported, in water buffaloes by Rahman *et al.* (2012) that a larger POF yielded a larger CL on day 5 and day 9 post ovulation and had a positive influence on successful pregnancy. Perry *et al.* (2005) also reported that a large POF is responsible for a large CL as well as a successful pregnancy. However, the ovulation rate was significantly lower in buffaloes that had POF size 9 to 12 mm (LPOF) as compared to other two groups whereas pregnancy rate was highest (51.43 per cent) in MPOF 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). This finding coincides with the results of Barile *et al.* (2007), Pandey *et al.* (2011), Neglia *et al.* (2011) and Rahman *et al.* (2012), showing that a larger POF was more competent to establish pregnancy in water buffaloes. Several reports in dairy cattle have also showed a positive association between POF size and subsequent conception rate (Lopes *et al.*, 2007; Perry *et al.*, 2007).

In the present study, the mean body condition score observed at the time of AI was  $2.7\pm 0.08$  (2-4). These observations support the earlier findings of Wongsrikeao *et al.* 1990 and Baruselli *et al.* 2001 who reported that the mean BCS of 3.3 had good ovarian activity during postpartum period. Buffaloes with high BCS (4-5) had exhibited early postpartum estrus compared to low BCS score (<3.5) on 1-5 scale. The analysis of the data revealed significant difference ( $P<0.05$ ) in the size of POF among the different groups of BCS.

On ultrasound scanning it was recorded that SPOF was present in 15, MPOF in 35 and LPOF in 20 buffaloes with mean diameters of  $10.53\pm 0.19$ ,  $13.36\pm 0.09$  and  $15.4\pm 0.13$  mm, respectively, ranging from 9 to 16 mm. These findings are concurring, with the findings of Baruselli (1997) in Murrah buffaloes ( $13.3\pm 1.8$  mm) and Presicce *et al.* (2005) in primiparous and pluriparous Meditterrean Italian buffaloes during postpartum estrus with a mean POF size of  $13.5\pm 0.8$  mm. 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  $13.5\pm 0.52$  to  $14.17\pm 1.08$  mm during the post-partum ovulations. Contrary to the present findings, Derar *et al.* (2012) reported the POF size as  $9.8\pm 0.32$  mm in Egyptian buffaloes which was much lower than the present observations. However, Taneja *et al.* (1996) and Neglia *et al.* (2007) reported the ovulatory follicle size as  $15.3\pm 0.03$  mm in non-lactating Murrah buffaloes and in Italian Mediterranean buffaloes ( $16.9\pm 0.16$  vs  $14.9\pm 0.25$  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 (Badinga *et al.*, 1994), feeding and management either under farm or field conditions.

More number of buffaloes showed intermediate (72.85 per cent) followed by weak (17.14 per cent) and intense (10 per cent) signs which is in accordance with the findings of Selvaraju *et al.* (2008). In the present experiment, only buffaloes presented for AI by the owners based on behavioural signs were included in the study, hence the number of buffaloes presented with weak estrus signs were less. In the present study, intensity of estrus was positively correlated with the BCS at the time of AI. These results were similar to those of Flores *et al.* (2007) and McKinniss (2008) who reported that the decline in the BCS, reduced the estrus response in dairy cattle. In the present investigation, the pregnancy rates were higher in intense estrus group. Similar findings were reported by Gunasekaran *et al.* (2007) who recorded 42.42 per cent conception rates in intense estrus group compared to the other two groups. The overall conception rate was 50 per cent. This was lower than the 63.63 per cent conception recorded by Verma *et al.* (2014) with accurate estrus detection methods on contrary in the same study Verma *et al.* (2014) in control group the conception was 41.93 per cent without concern to estrus detection. The conception of this study was, much higher than the earlier reports of Thirunavukkarsu 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. The mean values of BCS in pregnant and nonpregnant buffaloes were recorded as  $3.41\pm 0.12$  and  $2.95\pm 0.10$  score, respectively, with no significant ( $P>0.05$ ) difference in BCS between pregnant and non-pregnant buffaloes. Similar findings were also reported in Italian Mediterranean buffaloes (Terzano *et al.*, 2005). On the contrary, body

condition scores of less than 2.5 during breeding resulted in extremely low pregnancy rates (Shamsuddin *et al.*, 2001 and Jack *et al.*, 2010). The findings from the present study suggest that proper nutrition during the breeding season is necessary for acceptable reproduction.

## CONCLUSION

The reproductive performance viz. resumption of ovarian activity, less number of services per conception with a significant increase in breeding efficiency ( $P<0.01$ ) was observed in Graded Murrah buffaloes reared under rural conditions with a BCS of 3.50-3.99. Further it was concluded that physiological maturity of the follicle rather than its diameter influenced their fertility.

## ACKNOWLEDGMENTS

The authors are thankful to the Dean, SVVU, Tirupati (A.P) for providing necessary facilities to carry out the above research work.

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