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SUCCESSFUL MANAGEMENT OF MID CYCLE ESTRUS IN MURRAH BUFFALOES- A PRELIMNARY CASE STUDY

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ABSTRACT

Two pleuriparous Murrah buffaloes were presented with the history of artificial insemination 9 days back and since early morning animal exhibiting signs of estrus. Per rectal and ultrasonographical examination revealed presence of dominant follicle and CL on different or same ovary at the same time, uterus was toned and oedematous with scanty thick cervico vaginal discharge on back retching. To study the hormonal milieu serum progesterone estimation was done in both mid cycle estrus buffaloes and in other two animals with normal estrous cycle in same stage (Day 9 from the last date of A.I.). The diameter of CL was larger in buffaloes exhibiting midcycle estrus than that of true estrus. In this case study, during mid-cycle estrus secretion of estrogen was higher from dominant follicle of first follicular wave and due lower progesterone concentration from insufficient CL animal exhibit signs of estrum in mid lutel phase.

Keywords: *Bubalus bubalis*, buffaloes, mid cycle estrus, estrogen, progesterone, true estrus

INTRODUCTION

Buffalo had a major role in dairy industry in the developing countries of Asia and in India as it contributes 60% of total milk production. As India had maximum world population of buffalo, we produce two-third of the world's total buffalo milk and half of the world's buffalo meat (FAOSTAT, 2005). Estrus detection and correct time A.I. is a key factor which determines the success of any breeding program to improve reproductive performance in dairy animals (Diskin and Sreenan, 2000). Silent heat, late maturity, sub-estrus, irregular estrous cycle, seasonal breeding, summer anestrous, poor conception rate, late postpartum estrus, repeat breeding are the major constraints in buffalo reproductive performance and can be improved by efficient and correct time artificial breeding management (Madan, 1990).

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However, in past few years occurrence of mid-cycle estrus i.e. exhibition of signs of estrus during the mid-luteal phase of the estrous cycle is being reported as an aberration in the crossbred cows (Sood *et al*., 2009; Satheshkumar *et al*., 2014; Satheshkumar, 2018) which confused the farmer and veterinarian in the field conditions which causes increase in number of inseminations per conception and repeat breeding leads to economic loss to the farmer and milk industry (Sood *et al*., 2009). This communication presents the diagnosis and management of mid cycle estrus in two buffaloes.

MATERIALS AND METHODS

Two pleuriparous Murrah buffaloes at Livestock Farm Complex, Veterinary College Research Institute, Tirunelveli were presented to Veterinary Clinical Complex (VCC), Veterinary College Research Institute (VCRI), Tirunelveli and as per the breeding record animals were artificially inseminated nine days back and exhibiting the signs of estrus since early morning. Past breeding history suggested that Buffalo 1 (B1) calved four months back and inter estrus period was 23 days. Buffalo 2 (B2) calved five months back and inter estrus period was 22 days. On rectal examination, B1 had toned oedematous uterus with a dominant follicle and a hard CL like structure palpated on right ovary. On the other hand, B2 had oedematous and toned uterine horns with relaxed cervix and one follicle like structure on left ovary and CL like mass on right ovary.

Transrectal ultrasonographical examination revealed dominant follicle as an anechoic fluid filled cavity and corpus luteum as a hypo echoic mass with irregular boundaries. Biometric observations were recorded on Day 0, B1 revealed follicle (12.2 mm x 09.2 mm) and CL mass (16.90 mm x 14.70 mm) on right ovary (Figure 2.), whereas B2 revealed follicle on left ovary and CL on right ovary. To compare ultrasonographical findings in mid cycle estrus cases, two buffaloes (B3 and B4) exhibiting the signs of true estrus presented to VCC, VCRI, Tirunelveli for insemination were subjected to ultrasonographical examination which revealed a dominant preovulatory follicle and regressing CL (Table 1).

Blood was collected from B1 and B2 on the day animal exhibiting signs of mid cycle estrum and in B3 and B4 on Day 9 post A.I. to estimate serum progesterone (by using iChroma II Immunoassay Reader) and estrogen (at Lister Metropolis Laboratory, Tirunelveli) concentration.

RESULT AND DISCUSSION

On Day 2 of mid cycle estrus and true estrus animals repeated ultrasonography was performed which revealed anovulation in B1 and B2 on other hand B3 and B4 follicle had been ovulated.

In present two cases of mid cycle estrus, animal attendant was advised to bring the animals on next heat. On next heat Day 0, ultrasonography revealed 10.87x11.52 mm of follicle in B1 and 12.09x9.64 mm follicle in B2 with copious transparent cervico vaginal mucus discharge on back retching. A.I. was performed with standard procedure along with administration of Inj. Bruserelin Acetate 20 µg intramuscularly. Repeat A.I. was performed on Day 1. On Day 6, B1 had 16.89 x 17.62mm CL with 10.96 x 10.82mm follicle and B2 had 15.05 x 18.72 mm CL with 13.49 x 8.53 mm follicle subsequently Inj. Bruserelin

Acetate 20 µg was administered on Day 6. On day 40 ultrasonography confirmed pregnancy in both animals (Figure 1).

Sood *et al.* (2009); Satheshkumar *et al.* (2014); Satheshkumar (2018) reported that exhibition of signs of estrus in mid luteal phase of estrous cycle are called as mid cycle estrum in cross bred cows. In the present study, the case of mid cycle estrum in Indian buffaloes were recorded for the first time. As per Sood *et al.* (2009); Satheshkumar (2018) per rectal and ultrasonographical examination revealed presence of dominant follicle and corpus luteum on same (Figure 2) or different side ovaries and which is in accordance to our study.

The estrus behaviour and degree of uterine tonicity and nature of cervico vaginal mucus discharge correlated positively and directly proportional to the serum estrogen concentration (Lyimo*et al*., 2000; Noakes, 2001). In present study the cervico vaginal discharge during mid-cycle estrus was scanty and thick but during true estrus the discharge was transparent and copious.

In present study the average size of dominant follicle was approximately similar in mid cycle (10.73 mm) and true estrus (10.87 mm). On other hand the average size of CL was larger in mid cycle estrus (15.6 mm) animals than animals in true estrus (9.05 mm). These findings are in agreement with Satheshkumar, (2018) who reported non-significant difference in the size of dominant follicle and significantly larger CL in mid cycle group and the group of true estrus.

The hormonal estimation in present study revealed high estrogen (11.035 pg/ml) and low progesterone (2.67 ng/ml) serum concentration which is in agreement with Satheshkumar, (2018) who reported significant higher estrogen and lower progesterone serum concentration in cross bred cows (Table 2).

Chauhan *et al.* (1976) studied the incidence of gestational heat in buffalo. Sah (2002) concluded that due tothe intermediate decreased level of progesterone which was sufficient to maintain pregnancy but negative feedback to LH pulse cannot be maintained which in turn causes the maturation of dominant follicle, lead to the increase in the circulatory estrogen concentration subsequently animal exhibits the signs of estrus. In present study the animals exhibiting mid cycle estrus behaviour had higher estrogen secretion from dominant follicle and due to lower production of progesterone from corpus luteum or luteal deficiency causes the overriding of estrogen and animal exhibits the signs of estrus during mid-luteal phase followed by repeat breeding condition in these animals which is in agreement with Satheshkumar (2018); Satheshkumar *et al.* (2015); Satheshkumar *et al.* (2012).

In present study dominant follicle was not ovulated as during mid-cycle estrus as supra basal level (>1 ng/ml) of progesterone was sufficient to prevent ovulation by inhibiting the estrogen induced LH surge (Duchens *et al*., 1994). So A.I. during mid-cycle estrus would not lead to conception (Satheshkumar, 2018).

As per Willard *et al.* (2003) injection GnRH on day 5 or 11 and Hansen (2002) 11 to 14 post A.I. increased serum progesterone level which causes higher pregnancy rates by enhancing embryo survival rates by delaying the luteolytic mechanism (Mann *et al*., 1995) which occurs due to failed or delayed maternal recognition of pregnancy. Some reports suggests that GnRH agonist induces luteinization and (or) atresia of large follicles which enhances progesterone concentration (Dirandeh *et al*., 2014). In present study treatment with *Inj.* Bruserelin Acetate on

Table 1. Ultrasonographical biometry of dominant follicle and corpus luteum in mid cycle estrus and true estrus.

Ovarian structure	Type of estrus					
	Mid cycle estrus			True estrus		
	B1 (mm)	B2 (mm)	Average (mm)	B3 (mm)	B4 (mm)	Average (mm)
Dominant follicle	12.2x09.2	11.06x10. 49	10.73	11.35x10.39	10.38x10.85	10.87
Corpus luteum (CL)	16.90x14.70	15.96x14.83	15.6	09.97x08.35	09.26x8.63	9.05

Table 2. Serum estrogen and progesterone concentration in mid cycle estrus and true esrtus.

Hormone	Type of estrus					
	Mid cycle estrus			True estrus (on day 9 from A.I.)		
	B1	B2	Average	B3	B4	Average
Serum Estrogen concentration (pg/ml)	10.61	11.46	11.035	7.36	7.89	7.625
Serum Progesterone concentration (ng/ml)	2.24	3.11	2.67	5.68	6.14	5.91

Figure 1. Day 40, Gestation sac with anechoic amniotic cavity and hypoechoic foetal mass with CRL 22.1 mm in B1 buffalo.

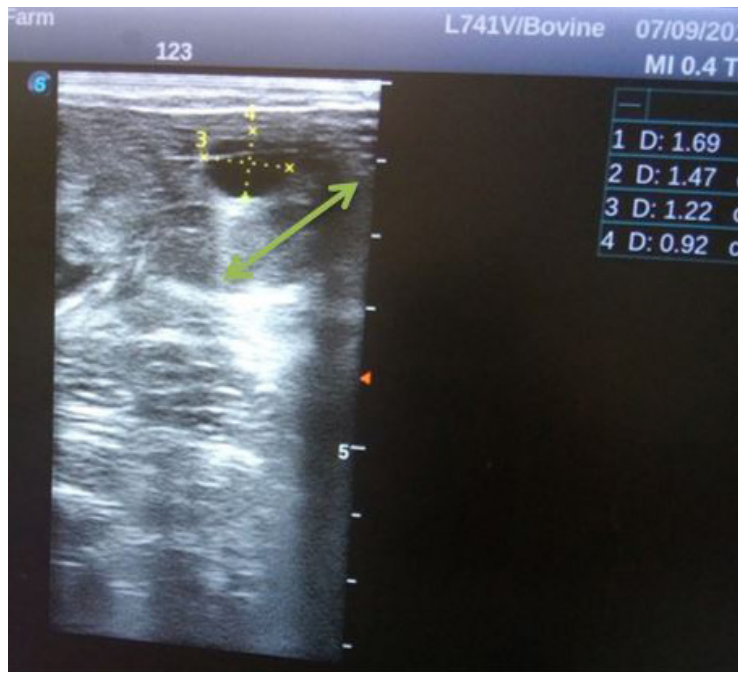

Figure 2. Biometry of corpus luteum and dominant follicle: hypoechoic mass (CL) D1:16.9 mm x D2:14.7 mm (Green colour arrow). An anechoic fluid filled cavity (dominant follicle) D3:12.2 mm x D4:9.2 mm in B1 buffalo.

Day 6 post A.I. (next cycle) induced ovulation or lutenisation of dominant follicle of first follicular wave. Satheshkumar *et al.* (2012) also suggested that the production of endogenous Progesterone can be increased by the administration of GnRH analogue on Day 6 post insemination which ovulate the dominant follicle and induce the formation of accessory CL in crossbred cattle.

CONCLUSION

The present study suggested that luteal deficiency during mid luteal phase of estrus cycle is the cause of mid cycle estrus in buffaloes, which could be diagnosed by transrectal and ultrasonographical examination. Animals with mid cycle estrus could be successfully treated with GnRH analogues on Day 6 post A.I. in next heat. This communication was first time reported as a successful management of mid cycle estrus in two Murrah buffaloes.

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