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Occurrence of Postpartum Anestrus and Estrus Behaviour in Hormonal Treated Buffaloes of Jabalpur

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ABSTRACT

The present study was conducted during June to December, 2022 at the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India to study the occurrence of postpartum anestrus and compare the intensity of estrus in anestrus and estrus induced buffaloes of Jabalpur (M.P.). A total of 120 postpartum buffaloes (5–10 years of age) reared under field conditions in the Jabalpur district of Madhya Pradesh were surveyed. The buffaloes with a history of not showing signs of estrus for 60 days or more postpartum were considered as open animals for the study of the occurrence of postpartum anestrus. Gynaeco-clinical examination was also carried out to know the status of ovaries (presence of follicle or corpus luteum). These animals were randomly divided into four groups (three treatments and one control group) and each group had 06 anestrus buffaloes. Group I, II and III were treated with CIDR plus Ovsynch, CIDR plus Cosynch and eCG plus Cosynch protocols, respectively, while the control group (Group IV) animals were not given any treatment. The results revealed that the occurrence of postpartum anestrus in buffaloes was 69.10%. The percentage of postpartum buffaloes with 60–90 days, 90–150 days and more than 150 days postpartum were recorded to be 25.30, 45.78 and 28.91%, respectively. Irrespective of treatment groups, the overall estrus intensity ranged from 58–92 with a mean estrus intensity score of 70.58±2.89. Estrus intensity scoring is a good tool to describe the intensity of estrus which has a positive relationship with fertility.

KEYWORDS: Estrus intensity score, gynaeco-clinical examination, occurrence

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1. INTRODUCTION

In India, agriculture is primarily a Crop-Livestock mixed production system where animal husbandry is a vital and integral part (Kumbhar et al., 2021). However, the average milk productivity of dairy animals in India is very low compared to other developed countries. Optimum reproductive performances of female populations in most cattle and buffalo breeding programs are important factors for reducing the cost of production (Meena et al., 2023). Buffalo is considered as most valuable livestock resource in Asian countries including India and occupies a critical niche in many agriculture systems providing milk, meat and work power (Lochan et al., 2020). Buffalo is the second largest source of milk supply in the world. Buffalo is considered a shy breeder and its reproductive performance is restricted because of various inherent problems like late puberty, delayed sexual maturity, silent heat, reproductive seasonality, high thermal stress, poor heat detection, postpartum anestrus and long intercalving interval resulting in economic losses to dairy farmers (Das and Khan, 2010; Kennady et al., 2018).

Anestrus is one of the most commonly occurring reproductive problems in buffalo affecting livestock productivity and economics to a great extent (Mujawar et al., 2019). Anestrus can be broadly defined as the considerable absence of estrus expression at the expected time frame (Perumal et al., 2021). Anestrus can be more precisely defined as functionless, quiescent ovaries and reproductive tracts. Thermal humid stress in buffaloes induces antigonadotrophic and antisteroidogenic functions (Shimamura et al., 1995) which in turn alter the estrous cycle and uterine environment leading to adverse effects in the uterine embryo development causing reproductive failure in buffaloes (Khan and Das, 2012), causes longer the post-partum anestrus (Megahed et al., 2006). Anestrus is caused by various factors including lack of sufficient nutrients, hormonal imbalance, lack of antioxidants, anemia, etc (Barile, 2005). A period of anestrus after parturition is a normal physiological event but becomes an abnormal condition if its duration exceeds its accepted average (Abraham, 2017). The postpartum period is the most crucial transitory phase in bovine life when various physiological, gynecological and biochemical changes occur (Perumal et al., 2013). The estrous cycle which ceases during pregnancy, usually resumes within two to three months of calving in buffaloes (Perera, 2011). However; only about 45% of Indian buffaloes resume cyclicity within 90 days postpartum and the rest 55% remain in anestrus for about 150 days (El-Wishy, 2007).

The incidence of anestrus has been reported to be higher in adult-aged buffaloes than the heifers (Bharkad and Markandeya, 2003). Gupta et al. (2015) reported a higher incidence of true anestrus (43.14%) followed by sub-estrus (33.2%) and other pathophysiological conditions (23.64%) in postpartum buffaloes of Jabalpur. Kalsotra et al. (2016) reported an incidence of 34.4% of postpartum anestrus in Murrah buffaloes of Jammu. Bisen et al. (2018) reported the highest percentage of incidence of sub-estrus (56%) followed by true anestrus (35.2%) and anestrus due to other physio-pathological conditions (8.8%) in postpartum dairy buffaloes from August 2015 to April 2016 in dairy buffaloes of Jabalpur. Kumar et al. (2019) reported an incidence of postpartum anestrus in buffaloes to be 33.4% in the Rewa district of M.P.

The delay in the onset of cyclicity of the estrous cycle and initiation of ovulation during the postpartum period in buffaloes constitute a major problem and results in long postpartum anestrus and delayed breeding with consequent serious economic losses in milk production and efficient reproduction (Bakr et al., 2015). The present study was planned to study the occurrence of postpartum anestrus and compare the intensity of estrus in anestrus and estrus induced buffaloes of Jabalpur (M.P.).

2. MATERIALS AND METHODS

The present study was carried out on 120 buffaloes reared under field conditions (organized and unorganized dairy farms) in different villages of Jabalpur from June to December, 2022. The location of work was Jabalpur situated at 23.17 °N latitude and 79.57 °E longitudes at 410.87 MSL (meters above sea level) in the southern part of the second agro-climatic zone, including Satpura plateau and Kymore hills, Madhya Pradesh. Jabalpur has a humid subtropical climate, typical of North-Central India. Summer starts in late March and lasts up to June. This is followed by monsoon season which lasts until early October, with a total precipitation of nearly 92.54 inches (2444.6 mm) due to South-west monsoon. Winter starts in late November and last until early march. The calving and breeding history of animals provided by the owners were recorded. The gynaeco-clinical examination was also carried out to know the status of ovaries (presence of follicle or corpus luteum) and reproductive tract (cervix and uterus) and findings were noted in the prescribed proforma. The buffaloes with a history of not showing the signs of estrus for 60 days or more in postpartum were selected for the study of the occurrence of postpartum anestrus.

For therapeutic management, 24 postpartum anestrus buffaloes were randomly selected from the 83 buffaloes found positive for anestrus during the survey. These animals were randomly divided into 4 groups (3 treatments and 1 control group) and each group had 6 anestrus buffaloes.

The animals of treatment groups were subjected to various synchronization protocols (Table 1) after 7 days of deworming as follows:

Table 1: Treatment protocols for different groups

Groups (n=6 animals /group)	Treatment regimen	Schedule
I: Modified Ovsynch protocol/CIDR +Ovsynch	Injection GnRH (Buserelin acetate 20 µg, I/M)+CIDR Implant	Day 0
	Injection PGF2α (Cloprostenol 500 µg, I/M)+Removal of CIDR Implant	Day 7
	Injection GnRH (Buserelin acetate 10 µg, I/M)	Day 9
	FTAI	Day 10 (24 hrs. after 2nd GnRH injection)
II: Modified Cosynch/ CIDR+Cosynch	Injection GnRH (20 µg, I/M)+CIDR implant	Day 0
	Injection $PGF_{2\alpha}$ (Cloprostenol 500 µg, I/M)+Removal of CIDR implant	Day 7
	Injection GnRH (Buserelin acetate 10 µg, I/M)+FTAI	Day 9
III: Modified Cosynch/eCG +Cosynch	Injection eCG (400 IU, I/M)	Day -3 (3 days prior to day 0)
	Injection GnRH (Buserelin acetate 20 µg, I/M)	Day 0
	Injection $PGF_{2\alpha}$ (Cloprostenol 500 µg, I/M)	Day 7
	Injection GnRH (Buserelin acetate 10 µg, I/M)+FTAI	Day 9
IV: (Untreated Control)	No treatment	AI on estrus

2.1. Estrus detection and breeding

Detection of estrus was carried out twice daily (morning and evening) by visual observations. Buffaloes at induced estrus were bred by natural service (NS) / artificial insemination (AI) on the fixed day of treatment protocols. The observations regarding induction and intensity of estrus after the end of treatment were recorded.

2.2. Estrus intensity score

It was numerically scored based on a proper weightage given for each of the parameters comprising estrus changes in the external and internal genitalia and behavioral expression. The estrus intensity was classified into intense, moderate and weak grade. Animals that failed to exhibit signs of estrus were considered silent estrus. For this purpose, the scorecard device of Sirmour (1999) was used.

2.3. Statistical analysis

The results of the incidence of anestrus and estrus induction were expressed in percentage. The data was analysed on the R platform (R Core Team, 2018) using the "dplyr" library. Data from different experiments were presented as Mean±SE. The results of estrus intensity were expressed in the number and percentage of animals.

3. RESULTS AND DISCUSSION

3.1. Occurrence

The occurrence of anestrus in postpartum buffaloes was recorded as 69.10% (83/120). The occurrence of postpartum anestrus in buffaloes was further categorized in terms of days postpartum. The percentage of postpartum anestrus in buffaloes with 60–90 days, 90–150 days and more than 150 days postpartum were recorded to be 25.30%, 45.78% and 28.91%, respectively (Table 2).

Table 2: Occurrence of postpartum anestrus buffaloes in terms of days postpartum

Postpartum (Days)	Buffaloes with postpartum anestrus	
	Numbers	Percentage
>150 days	24	28.91
90–150 days	38	45.78
60–90 days	21	25.30
Total	83	69.10

Kaurav et al. (2019) reported the incidence of postpartum anestrus in buffaloes in Rewa (M.P.) as 33.4%. The incidence of anestrus in the present study was found to be higher as compared to reports of Kumar et al. (2013), who reported the incidence of anestrus in the buffaloes with 25.84% in organized dairy farms of Jabalpur (M.P.) The higher incidence of anestrus might be because postpartum interval was considered as 60 days instead of 3 or 4 months (Kaurav et al., 2019). Another reason for the higher incidence of anestrus in the present study may be that the anestrus recorded was based on the history and gynaeco-clinical examination of the postpartum animals in the present study whereas only history of anestrus in survey

studies of other workers was recorded.

Wide variations do exist in terms of its percentage wise occurrence because of breed, sample size, criteria for consideration, etc. In the present study, a higher incidence might be due to the different criteria of considerations i.e. those buffaloes, failed to resume cyclicity within 60 days postpartum as against 75 days or more by other researchers, were taken into account.

In general, incidence of anestrus in buffaloes in India has been reported between 2.13%–67.11% (Kumar et al., 2014). In several studies, from the states other than Madhya Pradesh, incidence of anestrus in buffaloes was reported to be 9.18%–20.8% from Gujarat (Prajapati et al., 2005; Modi et al., 2011), 45.2%–54.38% from Uttar Pradesh (Kumar et al., 2007), 22.4% from Andhra Pradesh (Sriniwas et al., 2007), 31.98% from Punjab (Singh et al., 2003) and 35.71%–61.29% from Maharashtra (Hedaoo et al., 2008).

The period of postpartum anestrus is usually longer in buffalo. Buffaloes have lesser number of preantral and antral follicles, smaller sized preovulatory follicle and greater tendency of follicular atresia (Baruselli et al., 1997) which might be responsible for high incidence of anestrus in buffaloes. The higher occurrence of postpartum anestrus in buffaloes also might be due to difference in parity, season, environmental conditions, nutrition, managemental practices, lactation, age, breed and/or geographical area.

Under normal conditions, buffaloes resume cyclicity by 30–90 days (Perera, 2011), however; only about 45% of Indian buffaloes resume cyclicity within 90 days postpartum and rest 55% remain in anestrus for about 150 days (El-Wishy, 2007). Review of the available literature on controlled studies in dairy buffaloes revealed that first ovulation as detected by rectal palpation and progesterone analysis occurred between 28–71 and 24–55 days, respectively, after calving. Postpartum estrus was reported between 44 and 87 days in the studies by El-Wishy (2007) and Perera (2011).

Also, records available from Egypt, India and Pakistan indicate that only 34–49% of buffaloes showed estrus during the first 90 days after calving and 31–42% remained anestrus for more than 150 days (Sharma et al., 2014).

In swamp buffaloes both postpartum ovulation and estrus are more delayed than in dairy buffaloes. First postpartum ovulation is frequently followed by one or more short oestrous cycles (<18 days). Long anovulatory and anestrus periods due to prolonged inter-luteal phase were reported to occur after short cycles. Also, long anestrus periods due to cessation of cyclic activity (true anestrus) for 3 or more weeks and prolonged luteal activity for 28 days or more were described to occur in about 25 and 8–11% of the buffaloes, respectively, after the first or second ovulation.

3.2. Estrus behaviour and gynaeco-clinical symptoms in buffaloes

Reproductive organ status of buffaloes as visualized and confirmed on rectal examination at estrus, such as tumefaction of vulvar lips, cervico-vaginal discharge, vulvar mucus membrane colour, the extent of cervical relaxation, uterine tonicity, gonadal status, teaser mounting, bending of tail, micturition and bellowing in the induced/observed estrus buffaloes of group I, II, III and IV are summarized in table 3, 4, 5, 6 and 7 as follows:

3.2.1. Tumefaction of vulvar lips

In group I, the moderate and mild tumefaction of the vulva in 83.33% and 16.67% of estrus buffaloes was observed, respectively (Table 3). While in group II, the moderate and mild tumefaction of the vulva was observed in 66.67% and 33.33% of buffaloes in estrus, respectively (table 4). In group III, intense and moderate intensity tumefaction of the vulva in 50% of buffaloes in estrus were observed (table 5). While in the control group, the intense and moderate tumefaction of the vulva was observed in 50% of estrus buffaloes (table 6). Irrespective of any group, the intensity of estrus at induced estrus was observed as intense, moderate and mild tumefaction of vulva in 20%, 65% and 15% of animals, respectively. Mohan et al. (2010) found intense tumefaction of vulvar lips in induced estrus buffaloes. This may be due to increased estrogen level which plays an important role in tumefaction of vulvar lips at estrus.

3.2.2. Cervico-vaginal mucus discharge

In group I, the copious and moderate amounts of cervico-vaginal mucus discharge in 50 % and 16.67% of estrus buffaloes was observed, respectively (Table 3). In group II, 50% of estrus buffaloes showed moderate CVM discharge and 33.33% of estrus animals showed copious amounts of discharge (Table 4). In group III, 33.33% of estrus buffaloes showed copious and moderate amounts of CVM discharge, respectively (Table 5). While in the control group, 50% of buffaloes in observed estrus had a moderate amount of discharge (Table 6). At induced estrus and irrespective of estrus intensity, copious and moderate cervico-vaginal mucus discharge was observed in 35% and 40% of buffaloes while CVM discharge was absent in 25% of buffaloes (Table 7). Sharma et al. (2003) reported mucus discharge in 29% of buffaloes and per rectal examination, the flow of mucus from vagina was noted in 42% of cases. Ali et al. (2012) reported that cervico-vaginal mucus discharge was copious in 64%, moderate in 21% and absent in 14% of buffaloes.

Table 3: Reproductive organ status at different estrus intensities in treatment group I (CIDR+Ovsynch protocol)

Reproductive organs	Estrus intensity			Total 06
	Intense	Moderate	Weak	
	1 (16.67)	3 (50)	2 (33.33)	
1. Tumefaction of vulvar lips				
i. Intense	0 (0)	0 (0)	0 (0)	0 (0)
ii. Moderate	1 (100)	3 (100)	1 (50)	5 (83.33)
iii. Mild	0 (0)	0 (0)	1 (50)	1 (16.67)
2. Cervico-vaginal discharge				
i. Copious	1 (100)	2 (66.67)	0 (0)	3 (50)
ii. Moderate	0 (0)	0 (0)	1 (50)	1 (16.67)
iii. Absent	0 (0)	1 (33.33)	1 (50)	2 (33.33)
3. Vulvar mucus membrane				
i. Intense pink	1 (100)	2 (66.67)	2 (100)	5 (83.33)
ii. Pink	0 (0)	1 (33.33)	0 (0)	1 (16.67)
iii. Pale	0 (0)	0 (0)	0 (0)	0 (0)
4. Cervical relaxation				
i. External Os open and cervix fully relaxed	1 (100)	2 (66.67)	1 (50)	4 (66.67)
ii. External Os open and cervix partially relaxed	0 (0)	1 (33.33)	1 (50)	2 (33.33)
5. Uterine tonicity				
i. Intense	1 (100)	3 (100)	0 (0)	4 (66.67)
ii. Moderate	0 (0)	0 (0)	2 (100)	2 (33.33)
iii. Weak	0 (0)	0 (0)	0 (0)	0 (0)
6. Ovarian status				
i. Mature follicle	0 (0)	0 (0)	0 (0)	0 (0)
ii. Developing follicle	1 (100)	3 (100)	2 (100)	6 (100)
7. Teaser mounting				
i. Stands to mount	1 (100)	1 (33.33)	0 (0)	2 (33.33)
ii. Allow mount but escapes	0 (0)	2 (66.67)	2 (100)	4 (66.67)
iii. Teaser refuse to mount	0 (0)	0 (0)	0 (0)	0 (0)
8. Bending of tail				
i. Present	1 (100)	2 (66.67)	1 (50)	4 (66.67)
ii. Absent	0 (0)	1 (33.33)	1 (50)	2 (33.33)
9. Micturition				
i. Frequent	1 (100)	2 (66.67)	1 (50)	4 (66.67)
ii. Normal	0 (0)	1 (33.33)	1 (50)	2 (33.33)
10. Bellowing				
i. Present	0 (0)	1 (33.33)	0 (0)	1 (16.67)
ii. Absent	1 (100)	2 (66.67)	2 (100)	5 (83.33)

Figures in parenthesis indicate percentage

Table 4: Reproductive organ status at different estrus intensities in treatment group II (CIDR+Cosynch protocol)

Reproductive organs	Estrus intensity			Total 06
	Intense	Moderate	Weak	
	1 (16.67)	1 (16.67)	4 (66.67)	
1. Tumefaction of vulvar lips				
i. Intense	0 (0)	0 (0)	0 (0)	0 (0)
ii. Moderate	0 (0)	1 (100)	3 (75)	4 (66.67)
iii. Mild	1 (100)	0 (0)	1 (25)	2 (33.33)
2. Cervico-vaginal discharge				
i. Copious	1(100)	1(100)	0 (0)	2 (33.33)
ii. Moderate	0 (0)	0 (0)	3 (75)	3 (50)
iii. Absent	0 (0)	0 (0)	1 (25)	1 (16.67)
3. Vulvar mucus membrane				
i. Intense pink	1 (100)	1 (100)	1 (25)	3 (50)
ii. Pink	0 (0)	0 (0)	3 (75)	3 (50)
iii.Pale	0 (0)	0 (0)	0 (0)	0 (0)
4. Cervical relaxation				
i. External Os open and cervix fully relaxed	1 (100)	0 (0)	1 (25)	2 (33.33)
ii. External Os open and cervix partially relaxed	0 (0)	1 (100)	3 (75)	4 (66.67)
5. Uterine tonicity				
i. Intense	1 (100)	0 (0)	0 (0)	1 (16.67)
ii. Moderate	0 (0)	1 (100)	4 (100)	5 (83.33)
iii. Weak	0 (0)	0 (0)	0 (0)	0 (0)
6. Ovarian status				
i. Mature follicle	1 (100)	0 (0)	1 (25)	2 (33.33)
ii. Developing follicle	0 (0)	1(100)	3 (75)	4 (66.67)
7. Teaser mounting				
i. Stands to mount	1 (100)	0 (0)	1 (25)	2 (33.33)
ii. Allow mount but escapes	0 (0)	0 (0)	2 (50)	2 (33.33)
iii. Teaser refuse to mount	0 (0)	1 (100)	1 (25)	2 (33.33)
8. Bending of tail				
i. Present	1 (100)	1 (100)	3 (75)	5 (83.33)
ii. Absent	0 (0)	0 (0)	1 (25)	1 (16.67)
9. Micturition				
i. Frequent	1 (100)	1 (100)	3 (75)	5 (83.33)
ii. Normal	0 (0)	0 (0)	1 (25)	1 (16.67)
10. Bellowing				
i. Present	0 (0)	0 (0)	0 (0)	0 (0)
ii. Absent	1 (100)	1 (100)	4 (100)	6 (100)

Figures in parenthesis indicate percentage

Table 5: Reproductive organ status at different estrus intensities in treatment group III (eCG+Cosynch protocol)

Reproductive organs	Estrus intensity			Total 06
	Intense	Moderate	Weak	
	4 (66.67)	0 (0)	2 (33.33)	
1. Tumefaction of vulvar lips				
i. Intense	2 (50)	0 (0)	1 (50)	3 (50)
ii. Moderate	2 (50)	0 (0)	1 (50)	3 (50)
iii. Mild	0 (0)	0 (0)	0 (0)	0 (0)
2. Cervico-vaginal discharge				
i. Copious	2 (50)	0 (0)	0 (0)	2 (33.33)
ii. Moderate	1 (25)	0 (0)	1 (50)	2 (33.33)
iii. Absent	1 (25)	0 (0)	1 (50)	2 (33.33)
3. Vulvar mucus membrane				
i. Intense pink	4 (100)	0 (0)	0 (0)	4 (66.67)
ii. Pink	0 (0)	0 (0)	2 (100)	2 (33.33)
iii. Pale	0 (0)	0 (0)	0 (0)	0 (0)
4. Cervical relaxation				
i. External Os open and cervix fully relaxed	4 (100)	0 (0)	0 (0)	4 (66.67)
ii. External Os open and cervix partially relaxed	0 (0)	0 (0)	2 (100)	2 (33.33)
5. Uterine tonicity				
i. Intense	3 (75)	0 (0)	0 (0)	3 (50)
ii. Moderate	1 (25)	0 (0)	2 (100)	0 (0)
iii. Weak	0 (0)	0 (0)	0 (0)	3 (50)
6. Ovarian status				
i. Mature follicle	2 (50)	0 (0)	0 (0)	2 (33.33)
ii. Developing follicle	2 (50)	0 (0)	2 (100)	4 (66.67)
7. Teaser mounting				
i. Stands to mount	1 (25)	0 (0)	0 (0)	1 (16.67)
ii. Allow mount but escapes	3 (75)	0 (0)	0 (0)	3 (50)
iii. Teaser refuse to mount	0 (0)	0 (0)	2 (100)	2 (33.33)
8. Bending of tail				
i. Present	4 (100)	0 (0)	2 (100)	6 (100)
ii. Absent	0 (0)	0 (0)	0 (0)	0 (0)
9. Micturition				
i. Frequent	4 (100)	0 (0)	2 (100)	6 (100)
ii. Normal	0 (0)	0 (0)	0 (0)	0 (0)
10. Bellowing				
i. Present	3 (75)	0 (0)	0 (0)	3 (50)
ii. Absent	1 (25)	0 (0)	2 (100)	3 (50)

Figures in parenthesis indicate percentage

3.2.3. Vulvar mucus membrane

In group I, the vulvar mucus membrane was intensely pink in 83.33% of estrus buffaloes (Table 3), while in group II, 50% of buffaloes showed intense pink and pink mucus membrane of vulva (Table 4). The intense pink colour of the vulvar mucus membrane was observed in 66.67% of group III animals (Table 5) and pink mucus membrane was found in all control animals (100%) at the estrus phase (Table 6). At induced estrus and irrespective of estrus intensity, the vulvar mucus membrane was intense pink and pink in 60% and 40% of buffaloes, respectively (Table 7). The Reddening of vulvar mucosa was expressed in 65%–91% of buffaloes under different studies by Gill et al., 1973, Verma et al., 2014, Kumar et al., 2006 and Arunmozhi, 2009 (Purohit and Rao, 2018). The pink and congested mucosa is due to increased vascularity (hyperemia) under the influence of estrogen (Roberts, 1996) which is a supportive sign of estrus detection as reported by Nair (1987) and Dantre (1997).

3.2.4. Cervical relaxation

The cervical relaxation and opening of the external Os uteri were judged at induced estrus. In group I and III animals, the opening of external Os and full relxation of the cervix was observed in 66.67% of estrus buffaloes (Table 3 and 5), while only 33.33% of estrus buffaloes in group II animals showed the opening of external Os and full relaxation of the cervix (Table 4). In the control group, all animals showed partial opening and relaxation of cervix (Table 6). It was found that irrespective of the intensity of the estrus, the external Os was open and fully relaxed in 50% buffaloes and external Os was open and partially relaxed in 50% buffaloes (Table 7). Sharma et al. (2003) suggested that cervical relaxation should be considered as a better parameter for estrus confirmation rather than the uterine tone which was not as marked as in cows based on gynaecological investigation. The present study correlates with the views expressed by Roberts (1996) that the degree of relaxation of the cervix depends on the level of estrogen from the ovary and there is gradual relaxation from pro-estrus to estrus. hence all the buffaloes eliciting estrus had fully relaxed cervix and external Os open.

3.2.5. Uterine tonicity

The uterine tonicity is a sign of estrus and is of definitive help along with other characters to affirm estrus. It is a guideline to decide whether the animal is to be bred or not. In the present study, uterine tonicity during induced estrus irrespective of treatment groups and intensity of estrus was intense and moderate in 40% and 60% of buffaloes, respectively (Table 7). Buffaloes of Group I had intense and moderate tonicity of uterus in 66.67% and 33.33% of estrus buffaloes (Table 3), while in Group II and control animals, 83.33% and 100% of animals showed moderate uterine tonicity, respectively (Table 4 and 6). Buffaloes of Group III had intense and weak tonicity of uterus in 50% of animals at the estrus phase (Table 5). Intense uterine tone with coiling of the uterine horns was recorded in 52.98% of buffalo heifers and 64.46% of adult buffaloes in estrus (Polikarpus et al., 2014). Palpable turgidity of uterine horns has been mentioned as a consistent sign of estrus when present in conjunction with vaginal congestion and vulvar edema (Agrawal and Tomar, 1984, Danell et al., 1984, Sharma et al., 2013). Ali et al. (2012) in their study reported uterine tonicity was intense in 64.28%, moderate in 21.42% and weak in 14.28% of animals.

3.2.6. Ovarian status

The ovarian status observed on rectal palpation on the day of induced estrus for a developing or developed functional structure revealed that all the estrus animals of group I (100%), 66.67% of group II and III animals had developing follicles (Table 3, 4 and 5). The control group had mature and developing follicles in 50% of estrus buffaloes, respectively (Table 6). Irrespective of treatment groups, 25% of buffaloes had mature follicles and 75% of buffaloes had developing follicles (Table 7). Transrectal palpation of ovarian structures, however, has limitations of accuracy due to the smaller size of the ovaries and the ovarian structures in buffaloes. Palpation of the ovulatory follicle must be done with great care as undue pressure may result in the rupture of the follicle (Purohit and Rao, 2018).

3.2.7. Teaser mounting

Teaser mounting status was judged as whether the teaser was allowed to mount, he teaser was allowed to mount but later on animal escaped and the teaser refused to mount. In groups I and II, 33.33% of estrus buffaloes stands to be mounted (Table 3 and 4), while only 16.67% of animals allowed stands to be mounted in group III (Table 5). All control animals (100%) at the estrus phase allowed standing mount (Table 6). Overall teaser mounting was observed as teaser stand to mount in 35% of buffaloes, teaser allowed mounting but escaped mounting in 45% of buffaloes and 20% of buffaloes refused for teaser mounting (Table 7). Saini et al. (1988) reported that teaser bull mounted in all animals (100%) in PRID and PRID plus PMSG treated groups. Singh et al. (1983) reported that 80% of the treated animals showed a response to teaser bulls 24 hours after the end of treatment in norgestomet treated groups.

3.2.8. Bending of tail

Irrespective of treatment groups, the bending of the tail was judged as the presence or absence of bending of the tail in different estrus intensities in buffaloes. In groups

Table 6: Reproductive organ status at different estrus intensities in treatment group IV (Control)

Reproductive organs	Estrus intensity			Total 06
	Intense	Moderate	Weak	
	4 (66.67)	0 (0)	2 (33.33)	
1. Tumefaction of vulvar lips				
i. Intense	0 (0)	1 (100)	0 (0)	1 (50)
ii. Moderate	0 (0)	0 (0)	1 (100)	1 (50)
iii. Mild	0 (0)	0 (0)	0 (0)	0 (0)
2. Cervico-vaginal discharge				
i. Copious	0 (0)	0 (0)	0 (0)	0 (0)
ii. Moderate	0 (0)	1 (100)	1(100)	2(100)
iii. Absent	0 (0)	0 (0)	0 (0)	0 (0)
3. Vulvar mucus membrane				
i. Intense pink	0 (0)	0 (0)	0 (0)	0 (0)
ii. Pink	0 (0)	1 (100)	1 (100)	2 (100)
iii. Pale	0 (0)	0 (0)	0 (0)	0 (0)
4. Cervical relaxation				
i. External Os open and cervix fully relaxed	0 (0)	0 (0)	0 (0)	0 (0)
ii. External Os open and cervix partially relaxed	0 (0)	1 (100)	1 (100)	2 (100)
5. Uterine tonicity				
i. Intense	0 (0)	0 (0)	0 (0)	0 (0)
ii. Moderate	0 (0)	1 (100)	1 (100)	2 (100)
iii. Weak	0 (0)	0 (0)	0 (0)	0 (0)
6. Ovarian status				
i. Mature follicle	0 (0)	0 (0)	1 (100)	1 (50)
ii. Developing follicle	0 (0)	1 (100)	0 (0)	1 (50)
7. Teaser mounting				
i. Stands to mount	0 (0)	1 (100)	1 (100)	2 (100)
ii. Allow mount but escapes	0 (0)	0 (0)	0 (0)	0 (0)
iii. Teaser refuse to mount	0 (0)	0 (0)	0 (0)	0 (0)
8. Bending of tail				
i. Present	0 (0)	1 (100)	0 (0)	1 (50)
ii. Absent	0 (0)	0 (0)	1 (100)	1 (50)
9. Micturition				
i. Frequent	0 (0)	1 (100)	0 (0)	1 (50)
ii. Normal	0 (0)	0 (0)	1 (100)	01(50)
10. Bellowing				
i. Present	0 (0)	0 (0)	0 (0)	0 (0)
ii. Absent	0 (0)	1 (100)	1 (100)	2 (100)

Figures in parenthesis indicate percentage

Table 7: Overall reproductive organ status at different estrus intensities

Reproductive organs	Estrus intensity			Total 20
	Intense	Moderate	Weak	
	6 (30)	5 (20)	9 (45)	
1. Tumefaction of vulvar lips				
i. Intense	2 (33.33)	1 (33.33)	1 (11.11)	4 (20)
ii. Moderate	3 (50)	4 (66.67)	6 (66.67)	13(65)
iii. Mild	1 (16.67)	0 (0)	2 (22.22)	3 (15)
2. Cervico-vaginal discharge				
i. Copious	4 (66.67)	3 (60)	0 (0)	7 (35)
ii. Moderate	1 (33.33)	1 (20)	6(66.67)	8 (40)
iii. Absent	1 (33.33)	1 (20)	3 (33.33)	5 (25)
3. Vulvar mucus membrane				
i. Intense pink	6 (100)	3 (60)	3 (33.33)	12 (60)
ii. Pink	0 (0)	2 (40)	6 (66.67)	8 (40)
iii. Pale	0 (0)	0 (0)	0 (0)	0 (0)
4. Cervical relaxation				
i. External Os open and cervix fully relaxed	6 (100)	2 (40)	2 (22.22)	10 (50)
ii. External Os open and cervix partially relaxed	0 (0)	3 (60)	7 (88.88)	10 (50)
5. Uterine tonicity				
i. Intense	5 (83.33)	3 (60)	0 (0)	8 (40)
ii. Moderate	1 (16.67)	2 (40)	9 (100)	12 (60)
iii. Weak	0 (0)	0 (0)	0 (0)	0 (0)
6. Ovarian status				
i. Mature follicle	3 (50)	0 (0)	2 (22.22)	5 (25)
ii. Developing follicle	3 (50)	5 (100)	7 (88.88)	15(75)
7. Teaser mounting				
i. Stands to mount	3 (50)	2 (40)	2 (22.22)	7 (35)
ii. Allow mount but escapes	3 (50)	2 (40)	4 (44.45)	9 (45)
iii. Teaser refuse to mount	0 (0)	1 (20)	3 (33.33)	4 (20)
8. Bending of tail				
i. Present	6 (100)	4 (80)	6 (66.67)	16 (80)
ii. Absent	0 (0)	1 (20)	3 (33.33)	4 (20)
9. Micturition				
i. Frequent	6 (100)	4 (80)	6 (66.67)	16 (80)
ii. Normal	0 (0)	1 (20)	3 (33.33)	4 (20)
10. Bellowing				
i. Present	3 (50)	1 (20)	0 (0)	4 (20)
ii. Absent	3 (50)	4 (80)	9 (100)	16 (80)

Figures in parenthesis indicate percentage

I, II and III, 66.67, 83.33 and 100% of estrus buffaloes showed bending of the tail, respectively (Table 3, 4 and 5). In the control group, only 50% of animals at estrus showed prominent bending of the tail (Table 6). Overall, bending of tail was seen in 80% while 20% of buffaloes did not show bending of the tail (Table 7). Amonge et al. (1998) studied the breeding behaviour of Swamp buffaloes and reported raising tail as sign of estrus in 50.94% of buffaloes.

3.2.9. Micturition

Micturition was judged as whether the micturition was frequent or normal. The occurrence of frequent and normal micturition in the buffaloes in different estrus intensities was recorded. In group I, II and III, 66.67%, 83.33% and 100% buffaloes showed frequent micturition at the estrus phase (Table 3, 4 and 5). In the control group, 50% of animals showed frequent and normal micturition at the estrus phase (Table 6). The overall frequent micturition was present in 80% of buffaloes and 20% of buffaloes had normal micturition (Table 7). Rao and Kodagali (1982) reported frequent urination in 83.68% and absence in 16.32% of Surti buffaloes. However, Srivastava and Kharche (1985) observed micturition as very frequent, frequent and normal in 20.58%, 32.35% and 47.05% of buffaloes, respectively. Janakiraman (1988) reported that frequent micturition was the most reliable symptom of estrus in normal cycling buffaloes.

3.2.10. Bellowing

Bellowing was judged as the presence or absence of the bellowing in different estrus intensities in buffaloes. Bellowing was observed in 16.67% and 50% of estrus buffaloes of groups I and III, respectively (Table 3 and 5). While it was observed to be absent in all estrus animals of group II and control (Table 4 and 6). Irrespective of treatment groups, bellowing was present in 20% and absent in 80% of buffaloes (Table 7). Chohan et al. (1992) observed that bellowing was present in 52.27% Nilli-Ravi buffaloes and Amonge et al. (1998) observed 50.94% of animals exhibited bellowing. Hiremath (2013) reported bellowing was present in 68% of true anestrus buffaloes.

3.3. Estrus intensity score

Irrespective of treatment groups, the overall estrus intensity ranged from 58–92 with a mean estrus intensity score of 70.58±2.89. Statistical analysis revealed non-significant variation ($p \geq 0.05$) between the different groups for estrus intensity. Estrus intensity score of buffaloes ranged from 80–92, 68–74 and 58–64 in intense, moderate and weak estrus intensities at induced/observed estrus respectively, with a mean weighted score of 86.66±1.97, 70.80±1.02 and 62.22±0.77, respectively. There was a non-significant ($p \geq 0.05$) variation between estrus intensity scores at induced/observed estrus (Table 8). Out of the total 20 buffaloes, 30%, 25% and 45% of buffaloes were categorized in intense, moderate and weak estrus intensity, respectively.

Non-significant difference in the estrus intensity score between treatment groups clearly indicates the influence of CIDR plus Ovsynch, CIDR plus Cosynch and eCG plus Cosynch protocols. In group III, administration of PMSG three days before first GnRH resulted in development of follicle which was luteinized and transformed in to CL by first GnRH which acted as source of progesterone. The amount of progesterone secreted by this CL was sufficient enough to induce heat symptoms and additional supplementation of progesterone to improve heat intensity is not required in Co-synch plus protocol. However, in other treatment groups intra vaginal device (CIDR) performed the same function by providing exogenous progesterone. So, non-significant variation ($p \geq 0.05$) was observed between the different groups for estrus intensity.

Table 8: Estrus intensity at induced estrus in postpartum anestrus buffaloes

Character	Intense	Moderate	Weak
Range	80–92	68–74	58–64
Mean weighted score	86.66±1.97	70.80±1.02	62.22±0.77

4. CONCLUSION

Occurrence of postpartum anestrus in buffaloes was 69.10%. The percentage of postpartum buffaloes with 60–90 days, 90–150 days and more than 150 days postpartum were recorded to be 25.3%, 45.78% and 28.91%, respectively. Irrespective of treatment groups, the overall estrus intensity ranged from 58–92 with a mean estrus intensity score of 70.58±2.89.

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7. REFERENCES

Abraham, F., 2017. An overview on functional causes of infertility in cows. Journal of Fertilization: In vitro-IVF-Worldwide, Reproductive Medicine. Genetics and Stem Cell Biology 5(2), 1–6.

Agarwal, S.K., Tomer, O.S., 1998. Reproductive technologies in buffalo. Indian Veterinary Research

Institute, U.P. India.
Ali, R., Shukla, S.P., Nema, S.P., 2012. Hormonal induction of ovarian cyclicity and conception rate in postpartum anestrus buffaloes. Indian Journal of Field Veterinarian 7(4), 44–46.
Amonge, T.K., Sarkar, A.B., Bora, N.N., Roy, T.C., 1998. Study on the breeding behavior of swamp buffaloes under khuti system of management in Assam. Indian Veterinary Journal 75, 932–933.
Arunmozhi, N., 2009. Expression profile of estrogen, progesterone and oxytocin receptors during estrous cycle in buffaloes. PhD Thesis, Indian Veterinary Research Institute, Bareily, India.
Bakr, M.M., Noseir, M.B., Amrawi, G.A., 2015., Effect of exogenous progesterone in treatment of ovarian inactivity in the Egyptian dairy parturient buffalo-cows. Alexandria Journal for Veterinary Sciences 47(1), 191–200.
Barile, V.L., 2005. Reproductive efficiency in female buffaloes. Livestock Production Science 92, 183–194.
Baruselli, P.S., Mucciolo, R.G., Visintin, J.A., Viana, W.G., Arruda, R.P., Madureira, E.H., Oliviera, C.A., Molero-Filho, J.R., 1997. Ovarian follicular dynamics during the estrous cycle in buffalo (*Bubalus bubalis*). Theriogenology 47(8), 1531–1547.
Bharkad, G.P., Markandeya, N.M., 2003. Incidence of bovine anoestrum. Indian Veterinary Journal 80, 190–191.
Bisen, A., Shukla, S.N., Shukla, M.K., Mishra, A., 2018. Fertility response using timed insemination protocols in sub-oestrus buffaloes. Journal of Animal Research 8(3), 417–421.
Chohan, K.R., Chaudhary, R.A., Khan, N.V., Choudhary, M.A., Ahmed, M., 1992. Oestrus behaviour and fertility in normal cycling and oestrus synchronized buffaloes. Indian Dairy Science 45, 588–590.
Danell, B., Gopkumar, N., Nair, M.C.S., Rajagopalan, R.K., 1984. Heat symptoms and detection in Surti buffaloes heifers. Journal of Animal Reproduction 5(2), 1–7.
Dantre, U.K., 1997. Induction of oestrus in delayed pubertal crosssbred heifers treated with receptal and long acting steroid preparation. M.V.Sc. & A.H. thesis (Gynaecology & Obstetrics), Jawahar Lal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.).
Das, G.K., Khan F.A., 2010. Summer anoestrus in RDP have helped in buffalo–a review. Reproduction in Domestic Animals 45, 483–494.
El-Wishy, A.B., 2007. The postpartum buffalo II. Acyclicity and anestrus. Animal Reproduction Science 97(3-4), 216–236.
Gill, R.S., Gangwar, P.C., Kooner, D.S., 1973. Studies on the estrus behaviour in buffaloes. Indian Journal of Animal Science 43, 472–475.
Gupta, K.K., Shukla, S.N., Inwati, P., Shrivastava, O.P., 2015. Fertility response in postpartum anestrus buffaloes (*Bubalus bubalis*) using modified Ovsynch based timed insemination protocols. Veterinary World 8(3), 316–319.
Hedaoo, M.K., Khillare, K.P., Meshram, M.D., Sahatpure, S.K., Patil, M.G., 2008. Incidence of anoestrus in Surti buffaloes. Veterinary World 1(2), 46.
Hiremath, S.S., 2013. Controlled breeding and reproductive management in buffaloes using EAZI Breed CIDR. Buffalo Bulletin 32(2), 418–422.
Janakiraman, K., 1988. Some aspects of reproductive problems in buffaloes. Second World Buffalo Congress 2(1), 264.
Kalsotra, R., Sharma, U., Kumar, S., Kumar, S., 2016. Study of the incidence and factors affecting postpartum anoestrus in Murrah buffaloes around Jammu region. Buffalo Bulletin 35(4), 731–735.
Kaurav, P.S., Shukla, S.P., Bajaj, N.K., Manjhi, V.K., Thakur, R.K., 2019. Incidence of postpartum anoestrus and oestrus behavior and gynecological symptoms in hormonal treated postpartum anoestrus buffaloes. Journal of Animal Research 9(1), 153–157.
Kennady, V., Verma, R., Rahman, H., Yadav, H.P., Virmani, M., Kumar, D., Choudhiry, V., 2018. Factors influencing seasonal anestrus in buffaloes and strategies to overcome the summer anestrus in buffaloes. Biological Rhythm Research DOI: 10.1080/09291016.2018 1558740.
Khan, F.A., Das, G.K., 2012. Follicular characteristics and intrafollicular concentrations of nitric oxide and ascorbic acid during ovarian acyclicity in Water buffalo (*Bubalus bubalis*). Tropical Animal Health Production 44, 125–131.
Kumar, P.R., Shukla, S.N., Shrivastava, O.P., Purkayastha, R.D., 2013. Incidence of postpartum anestrus among buffaloes in and around Jabalpur. Veterinary World 6(9), 716–719.
Kumar, P.R., Singh, S.K., Kharche, S.D., Govindaraju, C.S., Behera, B.K., Shukla, S.N., Kumar, H., Agarwal, S.K., 2014. Anestrus in cattle and buffalo: Indian perspective. Advances in Animal and Veterinary Sciences 2(3), 124–138.
Kumar, R., Singh, R., Kumar, R., 2007. Control of anoestrus in buffaloes through locally available resources. Italian Journal of Animal Sciences 6(2), 659–662.
Kumar, R., Singh, U., Singh, I., Phogat, J.B., 2006. Estrus behavioral signs and its detection in hormonal treated summer anestrus buffaloes. Haryana Veterinarian 45, 57–60.

Kumar, S., Kumar, G., Gahalot, S.C., 2019. Anestrus in buffaloes and different treatment regimens. International Journal of Current Microbiology and Applied Sciences 8(3), 1162–1179.

Kumbhar, U.B., Charpe, B.K., Kumar, S., 2021. Bovine embryonic mortality with special reference to mineral deficiency, heat stress and endocrine factors: a review. International Journal of Bio-resource and Stress Management 12(1), 47–58. HTTPS:// DOI. ORG/10.23910/1.2021.2139a.

Lochan, S., Honparkhe, M., Cheema, R.S., Kumar, A., Ghuman, S.P.S., Brar, P.S., 2020. Ameliorating postpartum reproductive cyclicity using exogenous melatonin implant in water buffalo (*Bubalus bubalis*). Indian Journal of Animal Sciences 90(2), 181–184.

Meena, S.K., Saini, S., Gahan, M.K., Swami, P., Gurjar, D., Chandra, V., 2023. Effect of non-genetic factors on seminal traits of indigenous and crossbred bulls under semi-arid climatic conditions. International Journal of Bio-resource and Stress Management 14(2), 298–305.

Megahed, G.A., Alghandour, S.E.M., Othman, R.H., El-Zohery, F.A., 2006. The relationship between oxidants/antioxidants imbalance and postpartum fertility in cattle. Assiut Veterinary Medical Journal 52, 226–240.

Modi, L.C., Patel, P.A., Patel, S.P., Patel, G.G., Joshi, A.H., Suthar, D.N., 2011. Prevalence of reproductive problems in buffalo in Mehsana milk-shed area of Gujarat. International Journal for Agro Veterinary and Medical Sciences 5(4), 424–428.

Mohan, K., Kumar, V., Sarkar, M., Prakash, B.S., 2010. Temporal changes in endogenous estrogens and expression of behaviours associated with oestrus during the preovulatory period in Murrah buffaloes (*Bubalus bubalis*). Tropical Animal Health Production 42(1), 21–26.

Mujawar, A.S., Razzaque, W.A.A., Ramteke, S.S., Patil, A.D., Ali, S.S., Bhikane, A.U., Khan, M.A., Mogal, I.R., 2019. Estrus induction and fertility response in postpartum anoestrus marathwadi buffaloes using hormonal protocol along with vitamin E and selenium. International Journal of Livestock Resources 9(3), 289–296.

Nair, S., 1987. Biochemical studies in blood and cervico-vaginal mucus of normal and abnormal cycling crossbred cows. M.V.Sc. thesis (Gynaecology and Obstetrics), Jawahar Lal Nehru Krishi Vishwa Vidhyalaya, Jabalpur (M.P.).

Perera, B.M.A.O., 2011. Reproductive cycles of buffalo. Animal Reproduction Sciences 124, 1094–1099.

Perumal, P., De, A.K., Bhattacharya, D., Alyethodi, R.R., Bhowmick, S., Kundu, A., 2021. Effect of exogenous melatonin on endocrinological profiles, biochemical and antioxidant and oxidative stress profiles in post-partum anestrus Andaman local buffaloes (*Bubalus bubalis*) in tropical island ecosystem. Indian Journal of Animal Sciences 91(3), 206–213.

Perumal, P., Veeraselvam, M., Nahak, A.K., 2013. Herbal treatment in animal reproduction. International Journal of Bio-resource and Stress Management 4(3), 460–467.

Polikarpus, A., Grasso, F., Pacelli, C., Napolitano, F., De Rosa, G., 2014. Milking behaviour of buffalo cows: entrance order and side preference in the milking parlour. Journal of Dairy Research 81(1), 24–29.

Prajapati, S.B., Ghodasara, D.J., Joshi, B.P., Prajapati, K.S., Jani, V.R., 2005. Etio-pathological study of endometritis in repeat breeder buffaloes. Buffalo Journal 21(2), 145–165.

Purohit, G.N., Rao, T.K., 2018. Estrus detection in buffaloes. International Veterinary Information Service, Ithaca NY (www. ivis. org), Last updated.

Rao, N.M., Kodagali, S.B., 1982. Oestrus signs and significance for fertility in Surti buffaloes. Indian Journal of Animal Reproduction 2, 12–14.

Roberts, S.J., 1996. Veterinary Obstetrics and Genital Disease (Theriogenology). 2nd Edn., Edward Brothers, Michigan, U.S.A.

Saini, M.S., Galhotra, M.M., Sangwan, M.L., Razdan, M.M., 1988. Use of PRID in inducing estrus and its effect on the sexual behaviour of Murrah buffalo heifers. Indian Journal of Dairy Science 41(1), 40–42.

Sharma, R.K., Jerome, A., Purohit, G.N., 2014. Reproductive physiology of the male and female buffalo. Bubaline Theriogenology A, 5702, 0614.

Sharma, R.K., Singh, J.K., Singh, P., Dixit, V.B., 2003. Effect of oestrus behaviour on fertility in Murrah buffaloes. Intas Polivet 4(2), 152–155.

Sharma, V., Prasad, S., Gupta, H.P., 2013. Studies on physical and rheological properties of cervico vaginal mucus during early pregnancy in buffaloes (*Bubalus bubalis*). Veterinary World 6, 508–514.

Shimamura, K., Sugino, N., Yoshida, Y., Nakamura, Y., Ogino, K., Kate, H., 1995. Changes in lipid and antioxidant enzyme activities in corpora lutea during pseudopregnancy in rats. Journal of Reproduction and Fertility 105, 253–257.

Shrivastava, H.K., Kharche, K.G., 1985. Oetrus behaviour patterns in Murrah buffaloes. Cherion 14, 235–258.

Singh, C., 2003. Response of anoestrus rural buffaloes to the intravaginal progesterone implant during summer. Indian Journal of Animal Sciences 73(10), 1129–1130.

Singh, G., Singh, G.B., Sharma, R.D., Nanda, A.S., 1983.

Experimental treatment of summer anestrus buffaloes with Norgestomet and PRID. Theriogenology 19(3), 323–329.

Sirmour, S.K., 1999. Therapeutic and biochemical studies in anestrus cross bred heifers. M.V.Sc. thesis (Gynaecology and Obstetrics), Jawahar Lal Nehru Krishi Vishwa Vidhyalay, Jabalpur (M.P.).

Sriniwas, M., Naidu, K.S., Naidu, G.V., 2007. Incidence of reproductive disorders in rural buffaloes in Andhra Pradesh. Indian Veterinary Journal 84, 845–846.

Verma, K.K., Prasad, S., Mohanty, T.K., Kumaresan, A., Layek, S.S., Patbandha, T.K., Kantwa, S.C., 2014. Behavioural signs of estrus in different parity of Murrah buffaloes (*Bubalus bubalis*): a comparative study. Indian Journal of Animal Research 48(6), 620–624.