Research article

DOI: 10.48165/ijar.2023.44.01.9

The Indian Journal of Animal Reproduction

The official journal of the Indian Society for Study of Animal Reproduction

ACS Publisher www.acspublisher.com

Year 2023, Volume-44, Issue-1 (June)

ISSN 0970-2997 ***(Print)***

ISSN 2583-7583 ***(Online)***

Ultrasonography Assisted Estrus Detection Improves the Submission Rate in Prostaglandin Treated Sub-Estrus Murrah Buffaloes

Manas Kumar Patra*, Atul Singh Rajput, Jisna Kuttiyeri Sasidharan, Rehan Ahmad Raza, Thejaswini Meda Puttanarsappa, Athidi Lokavya, Atul Kumar Singh Tomar, Goutam Kumar Das, Subrata Kumar Ghosh and Gyanendra Kumar Gaur

ICAR-Indian Veterinary Research Institute, Izatnagar, U.P., 243 122

ABSTRACT

Sub-estrus is a condition in buffalo in which the ovarian picture and uterine changes suggest the presence of estrus and cyclicity, despite the absence of behavioural symptoms. The current study describes the effects of ultrasonography (USG)-aided estrus detection on submission and conception rate in sub-estrus Murrah buffaloes after a single shot of prostaglandin (PG) treatment. The study included 67 sub-estrus buffaloes between 2020 and 2021. The teaser bull was used to detect estrus, and the diameter of the pre-ovulatory follicle (POF) and corpus luteum (CL) was measured using transrectal USG. A single PG treatment induced behavioural estrus in 43.28% buffaloes (Group 1), while estrus was detected in 35.8% of sub-estrus buffaloes using sonographic examination (Group 2) that were otherwise remained undetected. The overall submission rate was 79.1 percent. The first-service conception rate was higher in Group 1 (55.2 vs. 33.3%), compared to Group 2. The CL diameter at pre-treatment was significantly larger in Group 1 buffaloes than in Group 2 buffaloes. However, the POF diameter was comparable during induced estrus. Furthermore, the time elapsed between induction and breeding in both groups was comparable. Furthermore, the CL size at pre-treatment, POF size at estrus, and time elapsed to breeding had no effect on conception. Thus, the use of USG improves the submission rate in PG administered sub-estrus buffaloes, resulting in a 45.3% overall first service conception rate. However, breeding time must be optimised in order to improve the conception rate and efficient reproductive management in sub-estrus buffaloes in the field.

Key words: Sub-estrus, Buffalo, Ultrasonography, Estrus induction, Prostaglandin, Preovulatory follicle size.

How to cite: Patra, M. K., Rajput, A. S., Sasidharan, J. K., Raza, R. A., Puttanarsappa, T. M., Lokavya, A., Tomar, A. K. S., Das, G. K., Ghosh, S. K., & Gaur, G. K. (2023). Ultrasonography Assisted Estrus Detection Improves the Submission Rate in Prostaglandin Treated Sub-Estrus Murrah Buffaloes.
The Indian Journal of Animal Reproduction, 44(1), 45–49. 10.48165/ijar.2023.44.01.9

*Corresponding author.
E-mail address: drmanas01@gmail.com (Manas Kumar Patra)
Received 16-05-2023; Accepted 17-07-2023

INTRODUCTION

Domestic water buffalo (*Bubalus bubalis*) contributes significantly to the Indian agricultural economy by providing milk, meat, and draught power. As mega dairy resource, buffaloes alone contributes around 45% to the total milk production in India (Basic Animal Husbandry Statistics, Govt of India, 2022). Despite their enormous production potential, buffaloes suffer from delayed sexual maturity, longer age at first calving, high vulnerability to summer stress, low ovarian reserve, luteal insufficiency and delayed ovulation. Restlessness, bellowing, and frequent urination are not consistently displayed by all buffaloes at estrus in the absence of a bull, and homosexual behaviour between females is rare (Perera, 2011). These factors greatly complicate breeding management in organised farm and field conditions. The key factor is a lack of overt estrus and silent ovulation in buffalo, which results in a longer service period and calving interval, causing huge economic losses to farmers.

Sub-estrus was found to be 54.5% in buffaloes who had not been in estrus for 60 days or more, and these buffaloes had a cyclic CL when examined per rectally or using ultrasonography (Rahman *et al.*, 2012). Estrus duration ranges from 4 to 64 hours (Ohashi, 1994), contributing to a low estrus detection rate (30-40%). A single or double dose of prostaglandin (PG) treatment induces estrus in these sub-estrus buffaloes (Brito *et al.*, 2002). In buffalo, the administration of natural or synthetic prostaglandins results in a significant decrease in progesterone concentration due to luetolysis (Markandeya and Bharkad, 2002). Simultaneously, on day 2 of PG injection, the dominant follicle (DF) increased in size from 0.97 ± 0.07 cm to 1.4 ± 0.09 cm, with a concomitant decrease in plasma progesterone and onset of estrus occurs in 3-4 days post injection (Day and Geary, 2005; Noseir *et al.*, 2014). However, the presence of a functional CL and the size of a growing follicle at the time of treatment have a significant impact on response to PG treatment (De Rensis and López-Gatius, 2007). The POF size could be measured using trans-rectal USG to improve treatment response and conception rates. Although repeated progesterone estimation could be used to confirm onset of estrus (Ribadu *et al.*, 1994), frequent sample collection and estimation procedures are prohibitively expensive and impractical for routine use. In this perspective, the present study describes the effect of USG-aided estrus detection on submission rate and conception rate following a single shot prostaglandin treatment in sub-estrus Murrah buffaloes.

MATERIALS AND METHODS

Experimental animals

The current study included 186 parous buffaloes maintained at the cattle and buffalo farm of the Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh. The study was conducted during October 2020 to March 2021. All of the animals appeared to be healthy and were kept in a loose house system under similar management conditions. Estrus detection was carried out by trained personnel using teaser (vasectomized) bull parading every morning and evening for at least 30 minutes.

Diagnosis of sub-estrus condition

The breeding records of all the eligible animals were examined. Buffaloes that had not been reported in estrus in the previous 60 days were examined trans-rectally, followed by ultrasonography at 7.5 mHz frequency (Exago, France). Buffaloes with a functional CL and no uterine pathology or prior estrus record were classified as sub-estrus (n=67) and included in the current study. The CL size was recorded for each buffaloes (Fig. 1a).

Treatment

All sub-estrus buffaloes were given a single shot of synthetic PG (Inj. Cloprostenol, 500g, i.m.) and were expected to show estrus in the next 3-5 days. Teaser bull parading was used to observe all of the buffaloes for behavioural estrus. On days 3, 4, and 5 following treatment, buffaloes that did not exhibit behavioural estrus were subjected to USG examination. Based on the observations, the buffaloes were divided into two groups: those showing natural estrus after PG administration (Group 1, n=29) and those detected in estrus using USG (Group 2, n=24). The buffaloes (n=11) that did not respond to natural estrus or USG were excluded from the study. In addition, three more buffaloes were excluded from the study because they had a missed heat and appeared to have developed CL on day 11/12 post treatment despite not being detected in the estrus.

Confirmation of estrus

USG examination was carried out on day 3-5 post treatment to confirm the estrus based on size of pre-ovulatory follicle, regressing CL and presence of mucus in the uterus.

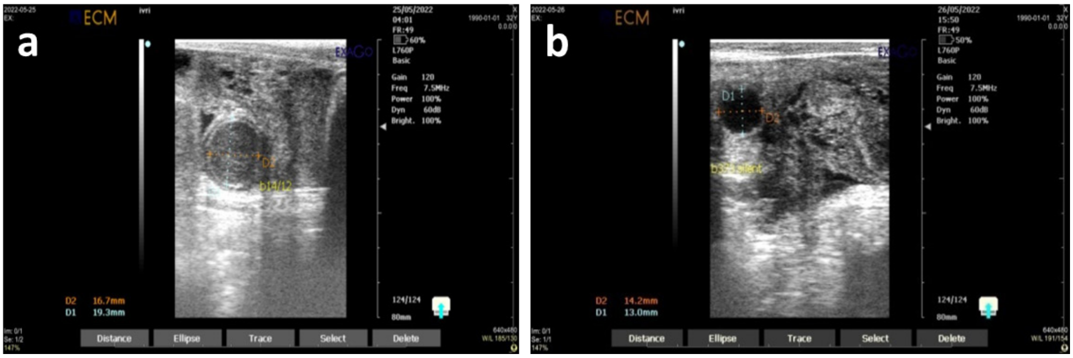

Fig. 1. Ultrasonographic observation of sub-estrus buffalo ovary. a. Matured corpus luteum (CL) present at the time of prostaglandin treatment, b. Preovulatory follicle (POF) developed at induced estrus.

The size of the pre-ovulatory follicle was determined (Fig. 1b). During induced estrus, cervico-vaginal mucus (CVM), vulvar lip swelling, and vaginal hyperaemia were also observed. All the buffaloes were artificially inseminated using frozen thawed semen from superior bulls on the a.m. and p.m. schedule. The time elapsed between PG administration and breeding was recorded.

Pregnancy diagnosis

A trans-rectal USG was used to confirm the pregnancy in all buffaloes on days 35-40 after breeding. Positive pregnancy was indicated by the presence of an amniotic vesicle containing a viable embryo.

Statistical analysis

The CL diameter, POF diameter, and time elapsed to breeding were compared using an independent't' test between buffaloes in Group 1 and 2. Furthermore, based on the retrospective categorization of buffaloes in to conceived and not-conceived independent t test was used to compare the CL diameter, POF diameter and time elapsed to breeding. Level of significance was set at $P<0.05$. The $\chi 2$ test was used to estimate the relationship between estrus response and conception. Graphpad Prism 6.0 was used to create the graphs.

RESULTS AND DISCUSSION

In the current study, we conducted screening on 186 Murrah buffaloes in a well-organized farm environment. The results revealed that 67 buffaloes (36%) were observed to be in sub-estrus. In a field study sub-estrus was reported up to 50% in buffaloes (Honparkhe *et al.*, 2008) and 47.5% of cattle using USG (Smith *et al.*, 1998). In current study estrus induction was observed in 43.3% of buffaloes in response to a single PG treatment using conventional estrus detection methods. However, with the use of USG estrus was detected in 35.8% of sub-estrus buffaloes, which would have been missed without detection and breeding. The overall 79.1% estrus induction was found in sub-estrus buffaloes using a single PG treatment by conventional estrus detection methods and USG. Dhaliwal *et al.* (1988) confirmed the finding, reporting an 87.5% estrus response rate in cyclic buffaloes. The proportion of buffaloes (43%) expressing behavioural signs at induced estrus was higher in the current study than in previous studies using cloprostenol (21.2 - 40%) and dinoprost, 27.2% (Singh *et al.*, 1984; Baruselli, 2001; Brito *et al.*, 2002). It is widely accepted that about 50% of estrus in buffaloes goes unnoticed despite rigorous monitoring, and 20.75% of buffaloes are inseminated outside of the fertile window (Kumaresan *et al.*, 2001). In the current study, the USG examination added 35.8% buffaloes for breeding and increased the submission rate up to 79.10%. Based on the presence of CL in the next examination after 10 days of treatment, only 4.5% of the buffaloes showed missed heat whereas 16.4% of the PG-treated buffaloes remained non-responsive. There were no discernible differences in CVM discharge, vulvar swelling, or vaginal hyperaemia (Table 1).

Table 1: Incidence of sub-estrus and estrus induction response in prostaglandin treated sub-estrus buffaloes.

Attributes	No of animals (%)
Incidence of sub-estrus	67/186 (36.02)
Observation of behavioural estrus	29/67 (43.28)
Estrus detected through USG	24/67 (35.82)
Overall estrus induction	53/67 (79.10)
Not responded into estrus	11/67(16.42)
Missed heat	3/67 (4.48)

USG examination is a reliable tool for determining CL diameter and functionality. In the current study, the diameter of CL at the time of PG treatment was greater (p=0.03) in Group 1 buffaloes that responded to natural estrus (19.7±0.61 mm) than in Group 2 buffaloes that were detected in estrus using USG (17±0.89 mm, Table 2. Although we did not measure serum progesterone levels in this study, higher progesterone production from a large CL during the luteal phase (Mishra *et al*., 2018) may be associated with better estrus response after induction. The time elapsed between treatment and induction varies greatly, ranging from 36 to 96 hours (Honparkhe *et al*., 2008) and 82.28± 6.51 hours in buffaloes (Dhaliwal *et al*., 1988). The time duration between treatments to breeding in the current study was 72.5 to 94.4 hr, and the mean duration was similar in both groups regardless of estrus response. Awasthi *et al*. (2007) found comparable results (3.2 ±0.27 days) after natural $PGF_{2\alpha}$ administration in buffaloes. The time elapsed from treatment to estrus response in buffaloes is largely determined by the size of follicles present in the ovaries. The presence of a dominant follicle at treatment could hasten the onset of estrus response (79.4±6.3hr) in buffaloes compared to those without a dominant follicle (85.3±7.1hr), where ovulation occurs primarily from the follicles of the next wave (Brito *et al*., 2002).

Table 2. Ovarian changes, time elapsed to breeding and first service conception rate in the prostaglandin treated sub-estrus buffaloes.

Attributes	Group 1 (n=29)	Group 2 (n=24)	P value
CL diameter at treatment (mm)	19.7±0.61 (18.3-21.1)	17±0.89 (15-18.9)	0.03
POF diameter at estrus (mm)	14.5±0.57 (13.1-15.9)	13.5±0.33 (12.8-14.2)	0.16
Time elapse to breeding (hr)	82.5±4.86 (72.5-92.4)	86±4.06 (77.6-94.4)	0.59
First service conception rate (%)	16/29 (55.17)	8/24 (33.33)	0.11

Group 1: Induced into natural estrus; Group 2: Estrus detected through USG

There was no significant difference in POF size at estrus between buffaloes in Group 1 and Group 2 (14.5±0.57 vs. 13.5±0.33 mm, p=0.16). The average POF diameter of the sub-estrus buffaloes ranged from 13.5±0.33 to 14.5±0.57 mm. Similarly, in cyclic buffaloes, the average POF diameter before ovulation was 14.09±0.40 mm (Riaz *et al*., 2018). Moreover, in the current study, the POF diameter had no significant effect on the onset of estrus response in either group. The time to breeding was also comparable in both groups (82.5±4.86 vs. 86±4.06 hr, p=0.59). However, the first service conception rate in natural estrus buffaloes, Group1 (55.17%) was relatively higher than in buffaloes detected in estrus via USG, Group 2 (33.33%, p=0.11). The conception rate of sub-estrus buffaloes at induced estrus was higher (55.2 vs. 33.3%) than those of USG-detected buffaloes (Fig. 2). The overall conception rate in the sub-estrus buffaloes was 45.3%, which was slightly lower than the rate in natural estrus buffaloes (51.42%, unpublished data). The relationship between estrus response and conception was non-significant (df=1), with an odds ratio of 2.46 for conception in the natural estrus group (Fig. 2). In the current study, the CL diameter at treatment, POF diameter at estrus, and time elapsed to breeding had no effect on conception status. However, the low conception rate in the USG detected buffaloes could be due to problems with delayed ovulation, and/or luteal insufficiency. The conception rate in cyclic buffaloes expressing natural estrus in the field ranged from 25% to 45% (Agarwal and Shanker, 1994) and 50% (Honparkhe *et al*., 2008). Furthermore, different estrus induction protocols resulted in a variable pregnancy rate ranging from 30.3% to 44.4% (Neglia, 2003) and 56.5 to 64.2% in buffaloes (de Araujo Berber *et al*., 2002). Furthermore, regardless of conception status, the CL diameter at PG treatment, POF diameter at estrus, and time elapsed to breeding were similar in sub-estrus buffaloes (Table 3).

Table 3. Ovarian changes, time elapsed to breeding in the sub-estrus buffaloes categorized based on the conception status.

Attributes	Conceived (n=24)	Not-conceived(n=29)	P value
CL diameter at treatment (mm)	18.2±1.33 (15.1-21.4)	18.1±0.67 (16.6-19.5)	0.89
POF diameter at estrus (mm)	13.6±0.51 (12.5-14.7)	13.9±0.33 (13.2-14.6)	0.60
Time elapsed to breeding (hr)	79.7±4.10 (71.2-88.1)	87.7±4.76 (78-97.5)	0.22

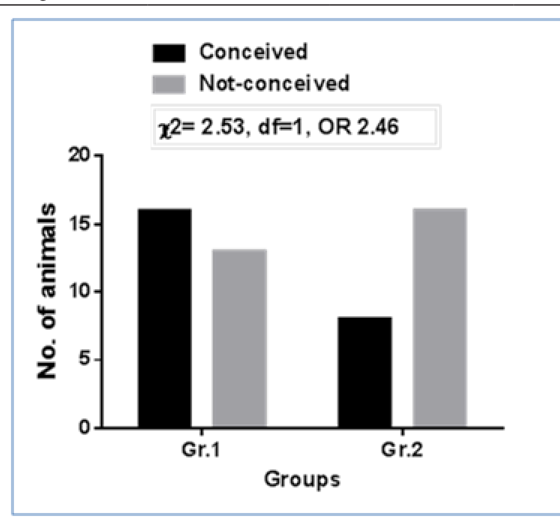

Fig. 2. Association of estrus response and conception in the prostaglandin treated sub-estrus buffaloes. Group 1: Induced into natural estrus, Group 2: Estrus detected through USG

CONCLUSIONS

From this study, it can be concluded that estrus detection using USG improves the submission rate by 35.8% in prostaglandin administered sub-estrus buffaloes, with an overall pregnancy rate of 45.3% under organised farm conditions. However, breeding time must be optimised in order to improve the conception rate further. As a result, USG-guided diagnosis of sub-estrus problems and monitoring of treatment response could improve reproductive efficiency of buffaloes in the field condition.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Agarwal, S.K. and Shankar, U. (1994). Annual Report, Livestock Production Research (Cattle and Buffalo). Published by Indian Veterinary Research Institute, Izatnagar, U.P., India.

Awasthi, M. K., Kavani, F. S., Siddiquee, G. M., Sarvaiya, N. P. and Derashri, H. J. (2007). Is slow follicular growth the cause of silent estrus in water buffaloes? *Anim. Reprod. Sci.,* **99**:258-268.

Baruselli, P.S. (2001). Control of follicular development applied to reproduction biotechnologies in buffalo. In: Proc. I Congresso Nazionale sull'Allevamentodel Bufalo, Eboli (SA), Italy.pp.128–146.

Basic Animal Husbandry Statistics. (2022). Published by Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry & Dairying. Government of India.

Brito, L.F.C., Satrapa, R., Marson, E.P., Kastelic, J.P. (2002). Efficacy of $PGF_{2\alpha}$ to synchronize estrus in water buffalo cows (*Bubalus bubalis*) is dependent upon plasma progesterone concentration, corpus luteum size and ovarian follicular status before treatment. *Anim. Reprod. Sci.,* **73**(1-2): 23-35.

Day, M.L., Geary, T.W. (2005). Handbook of estrus synchronization. Western Assoc Experiment Station Directors, WRP**014**:2-39.

De Araujo Berber, R.C., Madureira, E.H., Baruselli, P.S. (2002). Comparison of two Ovsynch protocols (GnRH versus LH) for fixed timed insemination in buffalo (*Bubalus bubalis*). *Theriogenology,* **57**(5): 1421-1430.

De Rensis, F. and López-Gatius, F. (2007). Protocols for synchronizing estrus and ovulation in buffalo (*Bubalus bubalis*): A review. *Theriogenology,* **67**(2): 209-216.

Dhaliwal, G.S., Sharma, R.D., Singh, G. (1988). Efficacy of prostaglandin $F_{2\alpha}$ administration for inducing estrus in buffalo. *Theriogenology,* **28:**1401–06.

Honparkhe, M., Singh, J., Dadarwal, D., Dhaliwal, G.S. and Kumar, A. (2008).Estrus induction and fertility rates in response to exogenous hormonal administration in postpartum anestrus and sub-estrus bovines and buffaloes. *J. Vet. Med. Sci.,* **70**(12): 1327–1331.

Kumaresan, A., Ansari, M.R., Sanwal, P.C. (2001). Assessment of accuracy of oestrus detection by progesterone assay in cattle and buffaloes. *Indian J. Anim. Sci., **71***(8): 758-760.

Markandeya, N.M., Bharkad, G.P. (2002). Effect of cloprostenol on conception rate during spring in sub-oestrus Murrah buffaloes. *Indian Vet. J.,* **79**:1205-1206.

Mishra, G.K., Patra, M.K., Sheikh, P.A., Teeli, A.S., Kharayat, N.S., Karikalan, M., Bag, S., Singh, S.K., Das, G.K., Krishnaswamy, N. and Kumar, H. (2018). Functional characterization of corpus luteum and its association with peripheral progesterone profile at different stages of estrous cycle in the buffalo. *J. Anim. Res.* **8**(3): 507-512.

Neglia, G., Gasparrini, B., Di Palo, R., De Rosa, C., Zicarelli, L. and Campanile, G. (2003). Comparison of pregnancy rates with two estrus synchronization protocols in Italian Mediterranean Buffalo cows. *Theriogenology,* **60**(1): 125-133.

Noseir, W.M.B., El-Bawab, I.E., Hassan, W.R., Fadel, M.S. (2014). Ovarian follicular dynamics in buffaloes during different estrus synchronization protocols. *Vet. Sci. Dev.,* **4**:25-29.

Ohashi, O.M. (1994). Estrous detection in buffalo cow. *Buffalo J.,* **10:**61–64.

Perera, B.M.A.O. (2011). Reproductive cycles of buffalo. *Anim. Reprod. Sci.,* **124**(3-4): 194-199.

Rahman, M. S., Shohag, A. S., Kamal, M. M., Parveen, N., and Shamsuddin, M. (2012). Application of ultrasonography to investigate postpartum anestrus in water buffaloes. *Reprod. Develop. Biol.,* **36**(2):103-108.

Riaz, U., Hassan, M., Husnain, A., Naveed, M.I., Singh, J., Ahmad, N. (2018). Effect of timing of artificial insemination in relation to onset of standing estrus on pregnancy per AI in Nili-Ravi buffalo. *Anim. Reprod.,* **15**(4):1231-1235.

Ribadu, A.Y., Ward, W.R. and Dobson, H. (1994). Comparative evaluation of ovarian structures in cattle by palpation per rectum, ultrasonography and plasma progesterone. *Vet. Rec.,* **135:** 452–457.

Singh, G., Singh, G.D., Sharma, S.S., Sharma, R.D. (1984). Studies on oestrus symptoms of buffalo heifers. *Theriogenology,* **21:** 849–858.

Smith, S.T., Ward, W.R. and Dobson, H. (1998). Use of ultrasonography to help to predict observed oestrus in dairy cows after the administration of prostaglandin $F_{2\alpha}$. *Vet. Rec.,* **142:** 271–274.3