

EFFECT OF LUTEAL REGRESSION ON CONCEPTION RATES IN POSTPARTUM ANESTRUS MURRAH BUFFALOES USING G48 AND G56 OVSYNCH PROTOCOLS

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ABSTRACT

The present study aimed to investigate the effect of luteal regression on the conception rates in postpartum anestrus graded Murrah buffaloes. Ovsynch protocols: G48 group (n=10) and G56 (n=10) graded Murrah buffaloes, both received a 20 µg dose of GnRH on day 0, followed by 500 µg of PGF2α on day 7. On day 9, a final dose of 20 µg of GnRH was administered 48 hours after PGF2α for G48 and 56 hours after for G56. Fixed-time artificial insemination (FTAI) was conducted at 16-21 hours after 2nd injection in both groups. The luteal regression rates in both the groups were evaluated by progesterone hormone (P4) assay at different days of treatment. The results showed that in both groups, the concentration of P4 differed significantly ($P<0.05$) between and within groups at different days of treatment. In conclusion, it was found that the G56 group (70.00 per cent) had a higher overall conception rate than the G48 group (50.00 per cent) due to better luteal regression.

Keywords: Buffalo, Postpartum anestrus, Ovsynch 48, Ovsynch 56, Luteal regression, Progesterone concentration and Conception rate

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INTRODUCTION

Three major contributors to poor reproductive efficiency in dairy animals are anestrus, repeat breeding syndrome and uterine infection. Postpartum anestrus is one of the key contributors to poor reproductive efficiency following parturition (Ohja *et al.*, 2017; Kumar *et al.*, 2021). Inadequate nutrition, high environmental temperature (Khan *et al.*, 2023) and low levels of steroid

hormones and pituitary gonadotropins in blood (Soliman *et al.*, 2024) will further aggravate its incidence. It was Pursley *et al.* (1995) who pioneered the use of hormone combinations paving the way for the present day ovsynch-48 protocol. Later, the ovsynch-48 protocol was modified in several ways to manage the luteal phase of estrous cycle with prostaglandins and progesterone hormones and also to control folliculogenesis and ovulation with combination of hormones for the enhancement of conception rate. The typical ovsynch-48 approach (Pursley *et al.*, 1995) involves a final GnRH treatment to synchronize the ovulation timing to eight hours after the second dose of GnRH injection and fixed time artificial insemination at any time between 0 and 24 hours showed identical conception rates. It was suggested that an increased time interval between prostaglandins and second GnRH in the ovsynch protocol had an impact on conception rate (e Silva *et al.*, 2023). Accordingly, extending the action of prostaglandins for more than 48 hours or delaying the second GnRH dose in ovsynch-48 protocol (also called as ovsynch-56) resulted in enhancement of conception rate in cows (Hassanein *et al.*, 2024) by causing development of dominant follicle and most importantly complete lysis of corpus luteum (EL Mahdy *et al.*, 2023). Unlike cattle, the estrous cycle in buffaloes demands some unique treatment approach due to their genetic makeup, management practices and environmental conditions. In light of the above, it is proposed to compare the efficacy of ovsynch-56 protocol over the

traditional ovsynch-48 on the luteal regression rates over conception rates.

MATERIALS AND METHODS

Animal selection and management

The present study was carried out in an organized dairy farm located in Vuyyuru Mandal, Krishna District, Andhra Pradesh, India. Twenty graded Murrah buffaloes, aged 2 to 8 years that calved normally, had no apparent health problems and were raised under the same management and feeding practises were chosen for the study. Selected buffaloes were screened and diagnosed for postpartum anestrus condition in which there were no signs of estrus for more than 60 days following parturition. This was done by the examination of the animals twice per rectally (P-1 and P-2), separated by 12 days apart to evaluate ovarian activity, specifically the presence or absence of a functional corpus luteum and follicular development.

Confirmation of anestrus

To confirm the anestrus condition in the selected buffaloes, blood samples were collected and serum was separated and stored at -20 °C for the estimation of progesterone hormone by ELISA method.

Hormonal therapy in ovsynch 48 and 56 group buffaloes

All the postpartum anestrus buffaloes in ovsynch 48 (n=10) and ovsynch - 56 (n=10) were administered with 20 µg of GnRH (Receptal, Intervet, Holland) intramuscularly

on day 0, 500 µg of Cloprostenol sodium (Vetmate, Vet care, India) intramuscularly on day 7. On day 9, buffaloes in the ovsynch - 48 group received 20 µg of GnRH (Receptal) intramuscularly 48 hours after the completion of Cloprostenol sodium administration. Whereas, those in the ovsynch - 56 group received 20 µg of GnRH (Receptal) 56 hours after the completion of Cloprostenol sodium administration. Fixed time AI (FTAI) was performed in both the groups at 16 to 21 hours after 2nd GnRH injection using frozen thawed semen of a fertile bull with minimum 50 per cent post thaw motility supplied by Andhra Pradesh Livestock Development Agency (APLDA). Pregnancy diagnosis was conducted by per rectal examination on 60th day following insemination.

Conception rate

The conception rate was calculated based on the percentage of buffaloes that became pregnant after their first and subsequent artificial inseminations, as determined by per-rectal diagnosis of pregnancy on day 60 after insemination.

Statistical analysis

All the data that was analyzed were presented as Mean±SEM. The "Chi square test" was also used to compare binary data on conception rates between two treatment groups. The unpaired t-test was used to compare single point measures such as progesterone.

RESULTS AND DISCUSSION

Diagnosis of anestrus

Serum progesterone levels were measured in both ovsynch-48 and ovsynch-56 groups during first (P-1) and second (P-2) preliminary examination to confirm anestrus status and the results are presented in table 1. The results revealed that the mean values from the two preliminary examinations were nearly similar and did not differ between two groups and the mean values were less than 0.5 ng/ml, indicating that all the buffaloes selected were in true anestrus condition.

Progesterone profile

The mean serum level of progesterone (P4) on different days of treatment are presented in table 1. The results showed that in both ovsynch-48 and ovsynch-56 the concentration of P4 differed significantly ($P<0.05$) between and within groups on different days of treatment. The mean serum progesterone concentration on the day of fixed time artificial insemination (FTAI) was significantly ($P<0.05$) lowest (0.65 ± 0.03 ng/ml) in ovsynch-56 when compared to ovsynch-48 protocol (0.88 ± 0.08 ng/ml). The findings of the present study are in agreement with the observations of Shaik (2014) who recorded a mean serum progesterone concentrations of 0.73 ± 0.11 ng/ml with ovsynch-48 protocol in postpartum anestrus buffaloes. Nevertheless, no such study has been carried out with ovsynch - 56 protocol. In the present study, a better luteal regression in ovsynch-56 animals was very well evidenced

Table 1: Serum progesterone (P4) concentration (ng/ml, Mean±SEM) on various days of treatment and conception rate in postpartum anestrus buffaloes subjected to ovsynch-48 (G48) and ovsynch-56 (G56) protocol

Group	Preliminary examination		Period of treatment					Conception rate
	1st day (P-1)	12 th day (P-2)	Day-0	Day-7	Day-9	Day-10	Day-60	
G48	0.44 ±0.00 ^a	0.43 ±0.01 ^a	0.43 ±0.01 ^a	3.95 ±0.10 ^{d**}	1.79 ±0.12 ^c	0.88 ±0.08 ^{b*}	2.81 ±0.38 ^c	50 (5/10) ^{NS}
G56	0.35 ±0.01 ^a	0.36 ±0.01 ^a	0.32 ±0.01 ^a	3.12 ±0.10 ^c	1.68 ±0.09 ^b	0.65 ±0.03 ^a	3.64 ±0.34 ^d	70 (7/10) ^{NS}
Overall								60 (12/20) ^{NS}

Values bearing different superscripts (a,b,c) along the row differ significantly ($P < 0.05$). Values bearing superscript (*) in a column differ significantly ($P < 0.05$). Values bearing superscripts (**) in a column differ significantly ($P < 0.01$). NS= Non Significance ($P > 0.05$)

where the second GnRH injection was delayed by eight hours as compared to conventional ovsynch - 48 protocol which could be inferred from the lower serum P4 value in the ovsynch-56 group buffaloes.

Conception rate

Overall conception rate was 60 per cent (12/20) from both the groups (table 1). Postpartum anestrus buffaloes subjected to ovsynch-56 protocol had an improved conception rate (70.00 per cent) over ovsynch-48 buffaloes (50.00 per cent). The improved conception rate observed in the present study could be due to a longer time gap between P4 decline and LH surge (56 hours vs. 48 hours) in the ovsynch-56 protocol,

which might have allowed a better oviduct environment for the survival and transport of gametes in the female reproductive tract (e Silva *et al.*, 2023). Increased interval between luteal regression and second GnRH might have caused an optimal LH surge required for ovulation (Sitko *et al.*, 2023). Further, increased period for luteolysis and better luteal regression in ovsynch-56 protocol (0.65 ± 0.03 ng/ml) compared to ovsynch-48 protocol (0.88 ± 0.08 ng/ml) might have resulted in better growth of pre-ovulatory follicle with increased exposure to estradiol under low level of circulating progesterone (Minela *et al.*, 2023). Furthermore, a delay in second GnRH could have improved interval from GnRH to LH surge, which might have caused

increase in the number of LH receptors on the pre-ovulatory follicle sufficient to trigger an ovulatory response (Belandria *et al.*, 2023). The delay in the second GnRH injection (56 hours after PGF2 α) might have also increased the size of the pre-ovulatory follicle that was responsive to exogenous GnRH, resulting in a higher conception rate (Fernandes *et al.*, 2022). When compared to ovsynch-48 with FTAI at 16 hours, GnRH at 56 hours might have allowed for the closest synchrony of ovulation in relation to artificial insemination i.e., ovulation happened 72 hours after PGF2 α (Allahyari *et al.*, 2023). Apart from the physiological benefits, the ovsynch-56 protocol offered better managerial convenience, particularly when the second GnRH injection was given in the afternoon on day 9, whereas the ovsynch-48 protocol did so in the morning had caused significant interference in routine farm operations and the timing of FTAI.

CONCLUSION

In conclusion, ovsynch-56 protocol is more advantageous over ovsynch-48 protocol in induction of estrus and synchronization of ovulation time for improved estrus response and better luteal regression rates thereby ensuring better conception rates in post-partum anestrus graded Murrah buffaloes. To validate these results, nevertheless, more research with a larger sample size is needed.

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