

METABOLIC, MINERAL AND ANTIOXIDANT LEVELS IN POST-PARTUM ANESTRUS BUFFALOES: A COMPARATIVE ANALYSIS OF OVSYNCH 48 AND OVSYNCH 56 PROTOCOLS

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ABSTRACT

The current study utilized 20 postpartum anestrus buffaloes maintained at an organized dairy farm in Vuyyuru, Krishna district to compare the metabolic (glucose), mineral (calcium) and anti-oxidant (superoxide dismutase) levels in ovsynch-48 (n=10, G48) and ovsynch-56 (n=10, G56) postpartum anestrus graded Murrah buffaloes. G48 group of buffaloes received 20 µg of GnRH on Day 0, followed by 500 µg PGF2 on Day 7, a final dose of 20 µg of GnRH was given 48 hours after PGF2α on Day 9. Whereas, G56 group of buffaloes received 20 µg of GnRH on Day 0, followed by 500 µg of PGF2α on Day 7, a final dose of 20 µg GnRH was given 56 hours after PGF2α on Day 9. In both groups, FTAI was performed at 16-21 hours interval after observing estrus. Results revealed that on different days of treatment, serum glucose and calcium levels did not differ significantly ($P>0.05$) in both treatment groups; however, Superoxide dismutase levels varied significantly ($P<0.05$) on different days of treatment in both groups. The results of the study parameters could play pivotal role in reproductive function and may provide insights into the underlying mechanism of anestrus.

Keywords: Graded Murrah buffalo, Postpartum, Anestrus, Ovsynch-48 and Ovsynch-56.

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INTRODUCTION

Post-partum anestrus poses a significant challenge in dairy buffalo management, leading to substantial economic losses due to delayed conception and extended calving intervals (Saini *et al.*, 2021). Studies in India have quantified the daily financial impact of anestrus in buffaloes at approximately Rs. 373/- which highlights the severity of the issue (Kumar *et al.*, 2013). The intricate interplay of metabolic cues and hormonal regulation,

particularly through the hypothalamo - pituitary - gonadal (HPG) axis underscores the complexity of reproductive cyclicity in buffaloes (McClure, 1965).

Low energy levels impede follicular development leading to follicular atresia and anestrus (Shakkarpude *et al.*, 2020).

Reduced energy intake during the post-partum period further disrupts hypothalamic GnRH secretion resulting in diminished luteinizing hormone levels and exacerbating anestrus (Richards *et al.*, 1987). Minerals such as calcium are crucial for cellular function and hormonal synthesis, with studies indicating significant differences in serum calcium levels between cyclic and acyclic buffaloes (Bohara and Devkota, 2009). Antioxidants play a vital role in reproductive processes by modulating follicular dynamics and other critical activities (Rizzo *et al.*, 2009). Enzyme antioxidants such as SOD and GPX act as a defense against reactive oxygen species, highlighting the importance of oxidative balance in reproductive health in bovine species (Anderson and Phillips, 1999; Fridovich, 1995).

Efforts to address post-partum anestrus globally involve the implementation of protocols aimed at inducing estrus and synchronizing ovulation along with advancements in dietary and management strategies. The pivotal work of Pursley *et al.* (1995) paved the way for protocols like ovsynch-48, which have shown promise in the improvement of conception rates in bovine species. Building upon this, studies have shown

that extending the action of prostaglandins beyond 48 hours or delaying the second GnRH dose (referred to as ovsynch-56) can significantly improve conception rates in cows (Stevenson and Britt, 2017).

Against this backdrop, the present study aimed to assess metabolic (glucose), mineral (calcium) and antioxidant (superoxide dismutase) levels in post-partum anestrus buffaloes undergoing ovsynch-48 and ovsynch-56 protocols.

MATERIALS AND METHODS

Experimental animals

The current investigation took place at a well-organized dairy farm situated in Vuyyuru Mandal, Krishna District, Andhra Pradesh. Twenty graded Murrah buffaloes aged between 2 to 8 years were carefully chosen for this study. These buffaloes were raised under identical management and feeding conditions and had shown no apparent health issues. The focus of the study was on post-partum anestrus, a condition where there were no signs of estrus for over 60 days of parturition. To assess ovarian activity, particularly the presence or absence of a functional corpus luteum and follicular development, all experimental animals underwent per rectal examination twice (P-1 and P-2), spaced 12 days apart.

Anestrus confirmation

Initially all the post-partum anestrus buffaloes were dewormed with Fensafe™ -I (3000 mg fenbendazole and 100 mg

ivermectin) blous. Later all the post-partum anestrus buffaloes in ovsynch 48 (n=10) and ovsynch 56 (n=10) were performed per rectal examination twice (P-1 and P-2), separated by 12 days apart and the acyclicity was confirmed by the progesterone assay. 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. Calbiotech progesterone ELISA kit (Catalogue number: PG362S) was used to estimate the progesterone hormone concentration in all of the experimental animals. Briefly the principle involves, upon adding samples to the working progesterone enzyme, conjugate and anti-progesterone-Biotin reagent to the streptavidin coated wells, the progesterone in test sample and progesterone HRP conjugate competes for the binding sites. Unbound progesterone and progesterone enzyme conjugate was washed off by washing buffer. TMB substrate addition produces color intensity which was inversely related to progesterone in the sample.

Ovsynch 48 and 56 protocols

Two days following the second per rectal examination, ovsynch protocol was initiated in both the ovsynch 48 (G48) and 56 (G56) simultaneously. G48 group of buffaloes received 20 µg of GnRH on Day 0, followed by 500 µg PGF2 on Day 7, a final dose of 20 µg of GnRH was given 48 hours after PGF2α on Day 9. Whereas, G56 group of buffaloes received 20 µg of GnRH on Day 0, followed by 500 µg of PGF2α on Day 7, a final dose of 20 µg GnRH was given 56 hours after PGF2α

on Day 9. In both groups FTAI was performed at 16-21 hours interval after observing estrus.

Metabolic, mineral and antioxidant parameters estimation in ovsynch 48 and 56 group buffaloes

For estimation of metabolic (glucose), mineral (calcium) and antioxidant (superoxide dismutase) parameters, blood collection was done on preliminary examinations (P-1 and P-2), day 0, 7, 9, 10 and 60 of treatment protocol period. Blood was collected in both ovsynch 48 and ovsynch 56 group postpartum anestrus buffaloes from the jugular vein with 18G disposable needles using clot activated vacutainers for serum and EDTA for plasma separation. Immediately after collection, the samples were transported to the laboratory in ice packed containers. Serum and plasma were separated by centrifugation at 3000 rpm for 15 minutes and stored in deep freezer at -20°C. Serum samples were used to estimate glucose (Trinder, 1969), calcium (Beeler and Catrou, 1983) and progesterone concentration, while plasma samples were used to determine superoxide dismutase levels (Madesh and Balasubramanian 1998).

Data Analysis

Statistical analysis was performed on the data collected from metabolic, biochemical and hormonal parameters using IBM SPSS statistics version 22. The unpaired t-test was used to compare single point measures such as progesterone. All the data that was analyzed were presented as Mean±SEM.

Table 1: Estrus response in ovsynch-48 (G48) and ovsynch-56 (G56) buffaloes

Parameter	ovsynch-48	ovsynch-56	t-value / chi-square value
Estrus response %	100 (10/10)	100 (10/10)	NS
No response %	-	-	NS

NS= Non significance (P>0.05)

Table 2: Estrus indicators in ovsynch-48 (G48) and ovsynch-56 (56) buffaloes

Indicators (%)		Ovsynch-48(G48)	Ovsynch-56(G56)
Frequent urination		100.00 (10/10)	100.00 (10/10)
Vulvar edema		100.00 (10/10)	100.00 (10/10)
Cervico Vaginal Mucus (CVM)		70.00 (7/10)	80.00 (8/10)
Bellowing		100.00 (10/10)	100.00 (10/10)
Uterine tone	Absent	-	-
	Mild	20 (2/10)	-
	Moderate	10 (1/10)	30 (3/10)
	High	70 (7/10)	70(7/10)

Table 3: Glucose concentration (mg/dl, Mean±SEM) on various days of treatment using ovsynch-48 (G48) and ovsynch-56 (G56) in postpartum anestrus buffaloes

Group	Preliminary examination		Period of Treatment				
	1st day (P-1)	12 th day (P-2)	Day-0	Day-7	Day-9	Day-10	Day-60
G48	55.79±2.24	54.56±2.19	56.42±1.28	54.35±2.57	56.17±1.24	56.93±1.94	54.98±1.89
G56	56.91±2.27	52.79±1.70	53.4±1.79	54.9±1.27	56.27±2.77	56.85±1.81	55.69±2.8

Table 4: Calcium concentration (mg/dl, Mean±SEM) on various days of treatment using ovsynch 48 (G48) and ovsynch-56 (G56) in postpartum anestrus buffaloes

Group	Preliminary examination		Period of Treatment				
	1st day (P-1)	12 th day (P-2)	Day-0	Day-7	Day-9	Day-10	Day-60
G48	10.58±0.68	11.33±0.58	10.72±0.84	10.13±0.59	10.19±0.45	10.16±0.43	10.07±0.45
G56	10.58±0.45	10.60±0.50	10.63±1.30	10.04±0.50	10.44±0.35	10.03±0.13	10.06±0.08

Table 5: Superoxide dismutase concentration (Units/μg of protein, Mean±SEM) on various days of treatment using ovsynch-48 (G48) and ovsynch-56 (G56) in postpartum anestrus buffaloes

Group	Preliminary examination		Period of Treatment				
	1st day (P-1)	12 th day (P-2)	Day-0	Day-7	Day-9	Day-10	Day-60
G48	3.46 ± 0.22	3.57 ± 0.30	3.79 ± 0.23	3.57 ± 0.316	3.47 ± 0.31	3.45 ± 0.24	3.45 ± 0.24
G56	3.81 ± 0.24 ^b	3.93 ± 0.22 ^b	3.12 ± 0.32 ^{ab}	2.11 ± 0.24 ^a	1.99 ± 0.19 ^a	2.62 ± 0.37 ^{ab}	2.96 ± 0.55 ^{ab}

Values bearing different superscripts (a,b,c) along the row differ significantly (P<0.05).

RESULTS AND DISCUSSION

Confirmation of anestrus in ovsynch-48 and 56 buffaloes

Serum progesterone levels were measured in both ovsynch-48 and ovsynch-56 groups during the first (P-1) and second (P-2) preliminary examinations to confirm anestrus status (Karen and Darwish, 2010). The mean values of serum P4 were 0.44 and 0.35 ng/ml in ovsynch-48 group and ovsynch-56, respectively on first preliminary examination (P-1) and 0.43 and 0.36 ng/ml in ovsynch-48 and ovsynch-56 group, respectively on the second preliminary examination (P-2). The results revealed that the mean values from the two preliminary examinations were less than 0.5 ng/ml, indicating that all the buffaloes selected were in true anestrus condition.

Estrous response

In the present study, ovsynch-48 and ovsynch-56 protocols were employed on post-partum buffaloes for estrous induction and synchronization of ovulation and both groups buffaloes equally responded with 100 % estrous response rate (Table 1).

Physiological indicators of estrus

According to the findings of present study, all the treated buffaloes of both groups exhibited frequent micturition, vulvar edema and bellowing, however estrus indicators such as cervico-vaginal mucus and uterine tonicity were more pronounced in ovsynch-56 group

thanovsynch-48 group (Table 2). Wherein the mucus discharge was noticed in 70 and 80% of buffaloes in G48 and G56, respectively and uterine tone was exhibited in three degrees of intensities viz., mild, moderate and high. The intensity of uterine tone was mild in 20% of buffaloes in ovsynch-48 group, moderate in 10 and 30% of buffaloes in ovsynch-48 and ovsynch-56 groups, respectively and high in 70% of buffaloes in both the groups.

Serum Glucose levels in Ovsynch 48 and Ovsynch 56 post-partum anestrus buffaloes

The mean serum glucose levels on different days of treatment are presented in Table 3. The results showed that there was no significant difference ($P>0.05$) between the two groups on different treatment days. In the present study, the mean serum glucose values obtained on different days of treatment in both the groups are in close agreement with the findings of Saikiran *et al.* (2020) and Maurya *et al.* (2021) who recorded the mean serum glucose levels as 54.17 ± 3.88 , 53.41 ± 6.63 and 56.97 ± 4.74 mg/dl, respectively in post-partumanestrus buffaloes. It is important to note that glucose is required for the biosynthesis of FSH hormone, a glycoprotein (Das and Kumar, 2018). Thus, nutrition had a direct impact on the reproductive system and its function, wherein it primarily involves endocrine system while negative energy balance show an impact on the diameter of the dominant follicle and corpus luteum, which could be one of the major cause of anestrus in the present study too (Hafez, 2000).

Serum Calcium levels in ovsynch 48 and ovsynch 56 post-partum anestrus buffaloes

The mean serum calcium levels on different days of treatment are presented in Table 4. The findings revealed that calcium levels in both groups on different days of study within the group, as well as between the groups were not significantly different ($P>0.05$). The higher serum calcium found in present study is consistent with findings of Kumar *et al.* (2015) in non-cyclic buffaloes, who reported the mean serum calcium level of 12.45 ± 0.63 mg/dl and Mane *et al.* (2018) findings, who reported the mean serum calcium level as 10.44 mg/dl. In fact, calcium levels alone may not have an impact on reproductive tract function, but the changes in the calcium and phosphorus ratio might impair reproductive cyclicity by preventing the hypophysis to release gonadotropins, resulting in a longer post-partum interval and delayed ovulation (Jayachandran *et al.*, 2013).

Plasma Superoxide dismutase (SOD) levels in ovsynch 48 and ovsynch 56 post-partum anestrus buffaloes

The mean plasma levels of SOD on different days of treatment are presented in table 5. The results revealed that within the ovsynch-56 group, plasma SOD levels differed considerably ($P<0.05$) on different days of therapy, wherein there was little variability within the ovsynch-48 group. The mean value of plasma SOD obtained in the current study is in agreement with findings of Chaudhary *et al.* (2015) and Saikiran *et al.* (2020) who reported the mean value SOD as

3.72 ± 0.18 and 3.20 ± 0.19 units/ μ g of protein, respectively. On the other hand, Ghosh *et al.* (2015) reported lower SOD levels (1.32 ± 19.48 units/ μ g of protein) in acyclic animals. The higher plasma SOD levels in the beginning of present study could be due to increased energy demand for milk production, which puts animal in a state of negative energy balance and causes oxidative stress. Further, an elevated free radicals generated by metabolic stress may explain the increased SOD levels in acyclic buffaloes (El-Razek *et al.*, 2019). The considerable decrease in plasma SOD levels in both the treatment groups (ovsynch-48 and ovsynch-56) on different days of treatment could be attributable to better managerial practices such as timely deworming, vaccination and provision of an adequate balanced ration to the animals.

In conclusion, our study successfully confirmed anestrus in both ovsynch-48 and ovsynch-56 buffaloes with serum progesterone levels consistently below 0.5 ng/ml. Analysis of serum glucose levels across treatment days showed no significant difference between the two groups which was in accordance with the previous research. Calcium levels also remained consistent throughout the study with no notable variations between groups. Notably within the ovsynch-56 group, plasma superoxide dismutase (SOD) levels exhibited significant differences across treatment days which is an indication of varied oxidative stress levels. Overall, our findings highlight the importance of management practices in the regulation of metabolic and reproductive functions in post-partum anestrus buffaloes.

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