RESEARCH ARTICLE

# Evaluation of Summer Stress Mitigation Strategies through Plasma Cortisol, Oxidative Markers and Conception Rates in Jaffarabadi Buffaloes *(Bubalus bubalis)*

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## Abstract

The current study was executed on postpartum (45-60 days) Jaffarabadi buffaloes from March to June 2023 (summer season), aimed at ascertaining how summer stress mitigation protocols affect conception rates and different biomarkers associated with oxidative stress of summer. The open animals were subjected to timed artificial insemination (TAI) Ovsynch protocol of estrus induction cum synchronization, and responded 24 buffaloes were randomly assigned to four different treatment/mitigation protocols or groups after AI (n=06 per protocol), *viz.,* the NSAID protocol, the antioxidant protocol, the $P_4$ protocol, and a control group. The percent conception rates at $35^{th}$ day (USG) and at $60^{th}$ day (per rectal palpation) in buffaloes under four groups were 50.00 and 0.00 %, 50.00 and 33.34%, 16.67 and 16.67%, and 16.67 and 16.67%, respectively, indicating embryonic loss between day 35 and 60 post-AI to the extent of 100% and 33.34% in NSAID and antioxidant treated groups, respectively, and nil in other two groups. The overall mean cortisol concentration was significantly ($p<0.05$) higher in $P_4$ supplemented than the control group, but it did not vary statistically between conceived, non-conceived, or animals with embryonic death groups or sampling days. The plasma MDA was significantly ($p<0.05$) lower in the antioxidant group and higher in the $P_4$ treated group. It also was significantly ($p<0.05$) higher in animals with embryonic mortality than those of the conceived and non-conceived groups. The TAC level was significantly higher in the antioxidant group and lower in the $P_4$ group. However, among the conceived, non-conceived, and animals with embryonic mortality, as well as across the sampling days, the mean TAC levels did not differ significantly. It was concluded that oxidative stress was the reason behind the embryonic losses in Jaffarabadi buffaloes, and that no treatment protocol was able to improve the summer conception rate.


**Key words:** Embryonic loss, Heat stress, MDA, Oxidative stress, Summer season, TAC.

*Ind J Vet Sci and Biotech* (2024): 10.48165/ijvsbt.20.3.26

## Introduction

Heat stress (HS) has become a significant problem for dairy animals as a result of climate change and global warming. Cattle and especially buffaloes have a negative impact of heat stress when it comes to reproduction (Roth, 2015). Summer/heat stress develops when an animal's capacity to regulate its body temperature through sweating and panting is disrupted by extremely hot, humid, or hot, dry weather. The Jaffarabadi buffaloes are more susceptible to heat stress due to their black coat coloration and lower sweat gland density. The breeding territory of Jaffarabadi buffalo includes the Saurashtra region of Gujarat state, where the summer temperature reaches up to 40-45°C. This high ambient temperature affects follicular growth, oocyte maturation, embryonic development, and implantation, all of which lead to early or late embryonic death/losses and thereby diminished reproductive efficiency. There was a decrease in the conception rate (CR) of cattle from 40% to 31% coincided with the increase in ambient temperature from 12.5°C to 35°C (Roth, 2015). Improvement in the fertility of Jaffarabadi buffalo during the summer season is possible by exploiting knowledge about how heat stress affects their reproductive process, and this can provide the information to improve the reproductive performance even under unfavorable or non-breeding season. However, the information on these aspects is still meagre in Jaffrabadi buffaloes from even in their home tract. Hence, this study was aimed to evaluate the summer stress mitigation strategies through plasma cortisol, oxidative markers and conception

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**How to cite this article:** Borakhatariya, D. N., Vala, K. B., Vijyeta, H. P., Karangiya, V. K., Singh, V. K., & Patbandha, T. (2024). Evaluation of Summer Stress Mitigation Strategies through Plasma Cortisol, Oxidative Markers and Conception Rates in Jaffarabadi Buffaloes (Bubalus bubalis). Ind J Vet Sci Biotech, 20(3), 132-137.

**Source of support:** Nil

**Conflict of interest**: None

**Submitted** 25/02/2024 **Accepted** 14/03/2024 **Published** 10/05/2024

rates in Jaffarabadi buffaloes (*Bubalus bubalis*) during summer season in their home tract, Junagadh.

## Materials and Methods

### Sample Collection

The study was carried out from March to June 2023 (summer season) at Cattle Breeding Farm, Kamdhenu University, Junagadh (Gujarat, India) following approval of the Institutional Animal Ethics Committee (IAEC) of the College (Protocol No.: KU-JVC-IAEC-LA-100-22). Data on various climatic variables for the experimental period were sourced from the Agrometeorological Cell, Department of Agronomy, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, and Thermal Humidity Index (THI) was calculated using the formula: THI= $(1.8 \times AT + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times AT - 26.8)]$, where, AT = air temperature (°C) and RH = relative humidity (%).

### Selection and Management of Experimental Animals

A total of 24 cyclic multiparous Jaffarabadi buffaloes, aged 4-8 years, with at least 45-60 days of voluntary waiting period (VWP) and sound reproductive status as found on per rectal gyneco-clinical examination were included in the study. All these animals were raised under uniform management of loose housing with free access to drinking water, and were given the seasonal fodder, hay and compounded concentrate, and wallowing of these buffaloes was discontinued.

### Experimental Design

All the experimental animals were subjected to timed artificial insemination (TAI) protocol of estrus induction cum synchronization, *i.e.,* Ovsynch protocol, where animals were administered with Inj. of 20 µg of GnRH analogue, *i.e.,* Buserelin acetate (Receptal, 5 mL, i/m, MSD Animal Health, Mumbai) on day 0, Inj. of 500 µg $PGF_2\alpha$ analogue, *i.e.,* dinoprost tromethamine (Lutalyse, 5 mL, i/m, Zoetis Pharma) on day 7, and second Inj. of 20 µg GnRH, i/m, on day 9, followed by FTAI twice at 24 and 48 h later. They were then randomly assigned to three different treatment protocols or groups, *viz.,* the NSAID (Nonsteriodal anti-inflammatory drug) protocol, the Antioxidant protocol, the $P_4$ protocol, and a non-treated Control group. Animals in group I (NSAID group) were given intramuscular injections of flunixin meglumine (Injection Megludyne, Flunixin Meglumine - 83 mg, Virbac Pharma) at a dose of 2.2 mg per kg body weight on days 14, 15, 16, and 17 after AI. Animals in group II (Antioxidant group) received subcutaneous injection of vitamin E and selenium (Injection Repronol, Vitamin E - 50 mg, Selenium - 1.5 mg, Cadila Pharma) at a dose rate of 1 mL per 50 kg body weight on the day of AI. In group III (Progesterone $P_4$ supplement), CIDR (1.38 gm progesterone in elastic rubber molded over a nylon spine, Zoetis) was implanted intra-vaginally on $4^{th}$ day after AI and kept *in situ* for 9 days, while, six Jaffarabadi buffaloes in group IV were kept as untreated Control post-AI. Animals were then regularly observed for the return of estrus around days 16-22 post-AI. In animals where estrus did not reappear, pregnancy was ascertained by trans-rectal ultrasonography (Mindray, Model: Z5 Vet USG) on day 35 after AI, and reconfirmation by per rectal palpation was done on day 60 post-AI. Conception rates found on day 35 and 60 post-AI, and embryonic losses noticed between these two intervals were recorded.

### Blood Sampling

Jugular blood samples (6-8 mL) were collected from all the 24 experiment animals in EDTA vacutainers on days 14, 16, 18, 20, 24, 27, 30, and 40 post-AI. The blood samples were centrifuged at 1372 g for 15 min, and the plasma separated was stored at -20° C with a drop of merthiolate (0.1%) until analyzed for plasma cortisol and oxidative stress markers (MDA: malondialdehyde; TAC: total antioxidant capacity). The plasma levels of cortisol were assessed on days 24, 27, 30, and 40 post-AI using an ELISA assay (MyBiosource, San Diego, USA), while plasma TAC and MDA were measured on all sampling days using chemical assays (HiMedia Laboratories Pvt. Ltd, Mumbai) as per the manufacturer's instructions.

### Statistical Analysis

Conception rates and embryonic losses in the various treatment groups were compared by Chi-square test, at 5% ($p<0.05$) and 1% ($p<0.01$) level. The data on plasma profile of cortisol, MDA and TCA were analyzed using two-way ANOVA, and the groups/treatment means were compared using Duncan's *post hoc* multiple range test, using Sigmaplot version 11.0 software (Systat Software Inc, USA).

## Results and Discussion

Mean daily values of the thermal humidity index (THI) varied from 75.0 to 83.0 during the summer months from March to June. Extreme heat stress was indicated by the THI beyond 72, which was quite high in May-June than in March-April during the study period.

### Conception Rates under Various Therapeutic Protocols

***NSAID Protocol (Grp-I):*** The conception rates obtained in Jaffarabadi buffaloes under NSAID protocol during the summer season were found to be 50.00% (03/06) at 35 days post-AI and zero % (00/06) at 60 days post-AI as compared to 16.67% each in control group. In this group there was 100% embryonic loss between days 35 and 60 post-AI. In earlier studies also NSAID treatment had a positive influence on pregnancy rate as evidenced by the higher pregnancy rates of 40.0 to 84.0 % in different breeds of cows and buffalo (Kasimanickam *et al.,* 2019; Damarany and Ghanem, 2020; Karasahin *et al.,* 2021). However, in other studies (Kaveh *et al.,* 2011; Spencer *et al.,* 2016) administration of NSAIDs did not improve the pregnancy rate. Erdem and Guzeloglu (2010) in contrast observed lower pregnancy rates (between days 31 and 38 after AI) in treated Holstein heifers than control group (24.3% vs. 52.0%).

***Antioxidant Protocol (Grp-II):*** Pursuant to the antioxidant protocol, the conception rate at 35 days post-AI was 50.00% (03/06) and at 60 days post-AI 33.34% (02/06) as compared to 16.67% each in control group. Further, 33.34% (01/03) of the animals which were pregnant at 35 days experienced embryonic losses in this group. This was of course lower than the embryonic loss experienced in NSAID group. The conception rate (50.00%) obtained in the current study during the summer season in Jaffarabadi buffaloes was higher than that of Kirdeci *et al.* (2021) (32.5%), but lower than that of Resum *et al.* (2018), and Smigoc *et al.* (2023) (64.7, 80%, respectively) in various breeds of cows after antioxidant treatment. However, Maldonado *et al.* (2017) and Likittrakulwong *et al.* (2022) found no evidence that antioxidant supplementation influenced the rates of conception in several breeds of cows.

***$P_4$ Protocol (Grp-III):*** The conception rates at both days 35 and 60 post-AI, in buffaloes supplemented with progesterone ($P_4$) during the summer season were found to be 16.67% (01/06) each, indicating that there was no loss of embryo during this phase. The much lower conception rate of 16.67% obtained in the current investigation utilizing the $P_4$ therapy was quite comparable to the findings of Monteiro *et al.* (2014) and Brozos *et al.* (2019), who found no impact of post-AI progesterone supplementation on the rate of conception in cattle. However, contrary to the current findings, higher conception rates (34.2 to 75 %) have been reported by many researchers (Borakhatariya *et al.,* 2017; Sharma *et al.,* 2021; Roth *et al.,* 2022).

***Control (Grp-IV):*** At day 35 and 60 after AI, the Jaffarabadi buffaloes in the untreated control group had a conception rate of 16.67% (01/06) each, indicating no loss of embryo between these days.

The study showed non-significant differences in conception rates between the treatment groups (Table 1). It was difficult to truly conclude on these results for limited number of animals covered in each group.

**Plasma Profile of Cortisol**

The overall mean plasma cortisol concentrations in Jaffarabadi buffaloes under NSAID, Antioxidant, $P_4$ protocols, and control group differed significantly with the highest value in $P_4$ protocol and the lowest in control group. Although there was no significant difference in plasma cortisol concentrations between the antioxidant and $P_4$ groups on different days post-AI, we did find that the NSAID protocol showed significantly higher mean plasma cortisol values (20.39±3.89 ng/mL) on day 24 post-AI, and lowest on day 40 (11.80±1.27 ng/mL) post-AI (Table 2). Furthermore, the values neither deferred statistically between conceived, non-conceived, and embryonic death groups nor between sampling days (Table 3).

The overall cortisol concentrations obtained during the summer months in Jaffarabadi buffalo across treatment groups were consistent with the results of numerous investigators in buffalo (Marai and Haeeb, 2010;

**Table 1:** Influence of different therapeutic protocols on conception rates (%) in Jaffarabadi buffaloes during summer season

| Treatment Groups | No. of Animals | Conception Rate (%) | | Embryonic losses (%) |
|---|---|---|---|---|
| | | Day 35 post-AI | Day 60 post-AI | |
| Grp-I (NSAID) | 06 | 50.00 (03/06) | 00.00 (00/06) | 100.00 (03/03) |
| Grp-II (Antioxidant) | 06 | 50.00 (03/06) | 33.34 (02/06) | 33.34 (01/03) |
| Grp-III ($P_4$) | 06 | 16.67 (01/06) | 16.67 (01/06) | 00.00 (00/01) |
| Grp-IV Control | 06 | 16.67 (01/06) | 16.67 (01/06) | 00.00 (00/01) |
| **χ2 (Fisher's Exact Test)** | | 2.87 | 2.38 | 5.38 |
| **P value** | | 0.49 | 0.87 | 0.014 |

Chi-square test between groups was non-significant (P<0.05).

**Table 2:** Plasma cortisol levels (ng/ mL) assessed in Jaffarabadi buffaloes on various days after AI during the summer season (Mean ± SEM)

| Treatment Groups | No. of Animals | Days from post-AI | | | | Overall |
|---|---|---|---|---|---|---|
| | | Day-24 | Day-27 | Day-30 | Day-40 | |
| Grp-I (NSAID) | 06 | 20.39±3.89$^{y}$ | 16.39±2.31$^{xy}$ | 18.56±2.31$^{xy}$ | 11.80±1.27$^{x}$ | 16.79±1.85$^{ab}$ |
| Grp-II (Antioxidant) | 06 | 15.37±3.20 | 14.19±0.80 | 13.98±1.75 | 16.21±1.98 | 14.94±0.52$^{ab}$ |
| Grp-III ($P_4$) | 06 | 16.22±1.34 | 14.64±1.67 | 18.63±1.00 | 18.00±1.26 | 16.87±0.90$^{b}$ |
| Control | 06 | 13.29±2.83 | 12.69±1.75 | 12.40±1.48 | 13.54±1.82 | 12.98±0.26$^{a}$ |

Figures bearing uncommon superscripts within a row (x, y) and a column (a, b) differ significantly at P<0.05

Rai *et al.,* 2023). Yet, in contrast to the current study, previous researches conducted by Dayal *et al.* (2017), and Yadav *et al.* (2022), noted much lower cortisol levels (4.25 to 7.5 ng/mL) in buffaloes during the summer, while Shenhe *et al.* (2018) and Li *et al.* (2021) recorded much higher levels (42.86-214.06 ng/mL). The current study showed that plasma cortisol levels during the summer season had barely any impact on the pregnancy outcome. Similarly, antioxidant supplementation had little effect on cortisol concentrations when compared to the control group.

## Plasma Profile of Malondialdehyde (MDA)

Among all of the protocols/groups, we found a significantly ($p<0.05$) lower mean plasma MDA in the antioxidant protocol, *i.e.,* 5.91±0.23 µM, which is suggestive of the positive/ beneficial effect of the antioxidant treatment on lipid peroxidation. It was significantly higher in $P_4$ treatment group (6.55±0.26 µM), which could be explained by the co-occurrence of heat stress and the intravaginal insertion of the CIDR device. Apart from this, the MDA concentration on day 27 post-AI was significantly higher in the $P_4$ treated group (6.82±0.58 µM) and lower in the NSAID group (4.99±0.52 µM). On the other hand, no statistically significant variation was noted between any treatment groups on other sampling days (Table 4). Further, the MDA levels of animals that conceived and those that did not, and those suffered embryonic death also differed significantly ($p<0.05$), being higher (6.93±0.22 µM) in embryonic mortality group than the conceived and non-conceived groups, nonetheless, latter two did not differ statistically (Table 5).

The elevated lipid peroxidation observed in this study, as indicated by the raised MDA levels, is consistent with the findings of numerous studies (Li *et al.,* 2021; Ayad *et al.,* 2020) in different breeds of cattle and buffalo during the summer season. Furthermore, the report by Celi *et al.* (2011), which found oxidative stress to be a contributing factor in Jersey cow embryonic mortality, is very similar to the significantly elevated levels of MDA found in the embryonic mortality group in the current investigation. Meanwhile, compared to the findings of Ayad *et al.*

**Table 3:** Plasma cortisol levels (ng/ mL) assessed in Jaffarabadi buffaloes on various days after AI during the summer season in conceived, non-conceived, and animals, who experienced embryonic mortality (Mean ± SEM)

| Status of Animals | No. | Days from post-AI | | | | Overall |
|---|---|---|---|---|---|---|
| | | Day-24 | Day-27 | Day-30 | Day-40 | |
| Conceived | **04** | 18.63±3.60 | 13.21±1.83 | 15.83±1.80 | 16.40±3.25 | **16.01±1.11** |
| Non-Conceived | **16** | 16.68±2.07 | 15.09±1.25 | 16.97±1.55 | 15.35±1.09 | **16.02±0.47** |
| Embryonic Mortality | **04** | 15.79±5.17 | 15.68±2.49 | 17.66±2.33 | 12.45±2.76 | **15.40±1.08** |

Values neither differ significantly across groups nor between samplings days ($p>0.05$).

**Table 4:** Mean (± SE) plasma MDA levels in Jaffarabadi buffaloes (µM) on different days after AI under different treatment groups during the summer season

| Treatment Groups | No. | Days post-AI | | | | | | | | Overall |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Day-14 | Day-16 | Day-18 | Day-20 | Day-24 | Day-27 | Day-30 | Day-40 | |
| Grp-I (NSAID) | 06 | 6.41±0.94 | 5.93±0.63 | 6.16±0.86 | 6.15±0.67 | 6.49±0.86 | 4.99±0.52$^{a}$ | 6.05±0.83 | 6.33±0.50 | **6.06±0.56$^{ab}$** |
| Grp-II (Antioxidant) | 06 | 6.61±0.29 | 6.04±0.67 | 5.71±0.47 | 6.43±0.57 | 5.41±0.36 | 5.75±0.54$^{ab}$ | 5.63±0.32 | 5.76±0.53 | **5.91±0.23$^{a}$** |
| Grp-III ($P_4$) | 06 | 6.54±0.79 | 6.72±0.47 | 6.01±0.33 | 6.30±0.35 | 6.27±0.33 | 6.82±0.58$^{b}$ | 6.85±0.32 | 6.88±0.30 | **6.55±0.26$^{b}$** |
| Control | 06 | 6.13±0.43 | 6.41±0.40 | 6.51±0.28 | 6.69±0.51 | 6.21±0.29 | 5.85±0.23$^{ab}$ | 6.2±0.42 | 6.91±0.22 | **6.37±0.23$^{ab}$** |

**MDA:** Malondialdehyde. Figures bearing superscripts (a, b) within a column differ significantly at $p<0.05$

**Table 5:** Mean (± SE) plasma MDA levels (µM) obtained in Jaffarabadi buffaloes on different days after AI during summer season in conceived, non-conceived, and animals that experienced embryonic mortality

| Status of Animals | No. | Days post-AI | | | | | | | | Overall |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Day-14 | Day-16 | Day-18 | Day-20 | Day-24 | Day-27 | Day-30 | Day-40 | |
| Conceived | 04 | 6.81±1.11$^{ab}$ | 5.64±1.01 | 5.43±0.26$^{a}$ | 5.63±0.41 | 5.17±0.40$^{a}$ | 5.49±0.67 | 6.36±0.67 | 5.64±0.79 | **5.77±0.19$^{a}$** |
| Non-conceived | 16 | 5.92±0.30$^{a}$ | 6.33±0.29 | 5.94±0.27$^{a}$ | 6.40±0.33 | 6.05±0.26$^{ab}$ | 5.89±0.31 | 6.10±0.22 | 6.61±0.20 | **6.16±0.09$^{a}$** |
| Embryonic mortality | 04 | 8.04±0.38$^{b}$ | 6.66±0.60 | 7.36±0.86$^{b}$ | 7.13±0.50 | 7.20±0.93$^{b}$ | 6.07±0.87 | 6.30±1.29 | 6.73±0.62 | **6.93±0.22$^{b}$** |

Figures bearing superscripts (a, b) within a column differ significantly at $p<0.05$.

**Table 6:** Mean (± SE) plasma profile of TAC in Jaffarabadi buffaloes (μM) on different days after AI under different treatment groups during the summer season

| Treatment group | No. | Days post-AI | | | | | | | | Overall |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Day-14 | Day-16 | Day-18 | Day-20 | Day-24 | Day-27 | Day-30 | Day-40 | |
| Grp-I (NSAID) | 06 | 74.83±15.50 | 60.56±10.18 | 51.68±9.79$^{a}$ | 67.04±17.26$^{ab}$ | 58.28±12.85 | 70.65±8.90 | 50.02±5.28 | 71.33±5.65 | **63.05±3.30$^{ab}$** |
| Grp-II (Antioxidant) | 06 | 74.16±14.21 | $^{xy}$90.9±15.61 | $^{xy}$98.76±23.74$^{b}$ | $^{y}$87.88±19.26$^{b}$ | $^{xy}$86.74±17.93 | 49.00±5.61 | $^{x}$60.02±14.63 | $^{xy}$70.15±8.94 | **77.20±5.99$^{b}$** |
| Grp-III ($P_4$) | 06 | 81.60±19.61 | $^{y}$90.08±38.04 | $^{x}$42.27±10.83$^{a}$ | $^{x}$41.55±5.02$^{a}$ | $^{xy}$61.25±20.74 | $^{x}$38.65±4.64 | $^{x}$40.69±3.96 | $^{xy}$55.92±8.44 | **56.50±7.03$^{a}$** |
| Control | 06 | 61.69±10.73 | 67.86±18.62 | 65.26±8.51$^{ab}$ | 78.32±14.03$^{ab}$ | 68.98±9.32 | 51.50±9.05 | 67.74±13.33 | 62.59±4.58 | **65.49±2.69$^{ab}$** |

**TAC:** Total antioxidant capacity. Figures bearing uncommon superscripts within the row (x, y) and column (a, b) differ significantly at $p<0.05$.

**Table 7:** Mean (± SE) plasma TAC levels (μM) recorded in Jaffarabadi buffaloes on different days following AI during the summer season in conceived, non-conceived, and animals that suffered embryonic mortality

| Status of Animals | No. | Days post-AI | | | | | | | | Overall |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Day-14 | Day-16 | Day-18 | Day-20 | Day-24 | Day-27 | Day-30 | Day-40 | |
| Conceived | 04 | 72.94±15.54 | 74.67±22.56 | 71.35±14.68 | 47.82±9.31 | 86.03±23.21 | 49.32±6.55 | 74.16±21.54 | 65.15±8.93 | **67.68±4.64** |
| Non-conceived | 16 | 70.80±9.22 | 83.56±15.41 | 64.21±11.40 | 69.10±9.44 | 66.38±9.44 | 49.07±5.17 | 51.32±5.58 | 65.42±4.85 | **64.98±3.87** |
| Embryonic mortality | 04 | 82.29±21.46 | 55.19±15.26 | 58.75±14.13 | 87.96±26.25 | 61.3±13.53 | 69.1±10.64 | 48.28±7.35 | 63.14±6.01 | **65.75±4.76** |

The plasma TAC levels neither differed significantly between sampling days nor between groups ($p>0.05$).

(2020), who reported MDA levels of 122.7±10.27 μM and 124.8±12.16 μM in pregnant and non-pregnant HF cows, respectively ($p<0.05$), the MDA levels obtained under the current study were much lower.

### Plasma Profile of Total Antioxidant Capacity (TAC)

While comparing the TAC results of the antioxidant and $P_4$ groups, a significant difference was found, with higher values (77.20±5.99 μM) in antioxidant group and the lower (56.50±7.03 μM) in $P_4$ group. The decreased lipid peroxidation and thus lower production of free radicals may account for the higher TAC in the antioxidant group. The higher oxidative stress shown by the elevated MDA levels is an explanation of the lower TAC in $P_4$ group. TAC levels of NSAID and control groups, on the other hand, did not differ, but levels in the antioxidant and $P_4$ groups differed significantly across different sampling days (Table 6). However, among the conceived, non-conceived, and embryonic mortality groups, it neither varied significant between the groups nor across the sampling days (Table 7).

The results of this investigation showed that the TAC levels of the animals, which experienced embryonic death and the animals that were conceived were similar. This finding contradicts that of an earlier study conducted by Abdoon *et al.* (2020), who observed decreased enzymatic antioxidant activity in oxidative stress, which in turn leads to embryonic death. However, the published results by Ayad *et al.* (2020), which also demonstrated that pregnant animals had lower enzymatic antioxidant activity than non-pregnant animals, are in contrast with the identical TAC levels seen in the conceived and embryonic death groups. However, it was difficult to track pertinent data for the debate regarding the overall antioxidant level in relation to summer months' pregnancy outcomes.

## Conclusion

The findings of current experiment revealed that neither of the three protocols, *viz.*, NSAID, Antioxidant, and $P_4$, augmented the pregnancy rate during the summer season in Jaffarabadi buffaloes. Supplementing Jaffarabadi buffaloes with vitamin E and selenium as an antioxidant at TAI reduced embryonic losses compared to NSAID. The post-AI progesterone supplementation (CIDR) had a negative impact on plasma levels of cortisol perhaps for intravaginal implant, while antioxidant supplementation had a minimal impact on cortisol levels. Moreover, the $P_4$ treatment significantly raised MDA levels, while the antioxidant treatment resulted in a significant drop. Animals with embryonic mortality had plasma MDA levels significantly higher, which could be the reason behind the summer embryonic losses. Antioxidant supplementation led the plasma total antioxidant capacity (TAC) to rise significantly, whereas $P_4$ treatment caused the TAC levels to drop significantly. The TAC levels of conceived, non-conceived, and animals with embryonic death were statistically similar, indicating that insufficient antioxidant capacity may not be the cause of the summer-time embryonic losses.

## Acknowledgements

Authors would like to express sincere gratitude to the Research Scientist, Cattle Breeding Farm, Kamdhenu University, Junagadh as well as Principal, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, for their generous support and facilities provided for this research work.

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