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Periodic critical micro-nutrients supplementation affects reproduction performance in peri-parturient dairy buffaloes

VIKAS JYANI[1], VISHAL MUDGAL[2]✉, MEENAKSHI GUPTA[1] and RAKESH KUMAR SHARMA[2]

ICAR-Central Institute for Research on Buffaloes, Hisar, Haryana 125 001 India

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The peri-parturient period is the period around parturition and plays a crucial role in affecting the health and subsequent performance of dairy animals. Animals get exposed to drastic physiological changes and metabolic stress during this period, and how do the animals face this challenge will decide their future performance. The peri-parturient period involves long-term adaptations to a change in state, such as from being non-lactating to lactating, and involves a series of changes in metabolism that allows an animal to adapt to challenges of the altered state. It has been hypothesized that the involvement of oxidative stress during the periparturient period is the etiology of some diseases and disorders in a dairy animal and thus compromised reproduction. The normal reproductive functions of livestock are closely associated with their nutritional status (Suharyono *et al.* 2018). Micronutrients play a very crucial role in the animal body besides the maintenance of normal health (Kurutas 2016, Singh *et al.* 2020).

Micronutrients deficiencies, imbalances, and toxicity of certain mineral elements may cause reproductive disorders as micronutrients play an important role in the health and reproduction of the livestock (Sharma *et al.* 2007, Mudgal *et al.* 2014, Vipin *et al*. 2020). Body micro-nutrients status and reproductive functions have a direct relationship (Wilde *et al.* 2006). Micronutrients including minerals like Cu, Zn, Co, Se, Cr and vitamins like vitamin E, vitamin A and niacin are the target of this study which activate several enzymes responsible for various biochemical functions and some are an integral part of enzymes/hormones. Therefore, keeping the aforesaid facts in view, supplementation of critical micronutrients (CMN) was carried out during the peri-parturient period to study the effect on reproduction status of buffaloes.

The study was performed on Murrah buffaloes maintained at Animal Farm Section of ICAR-Central Institute for Research on Buffaloes, Hisar, Haryana, India, from September to April, when humidity varied from 39–72% and ambient temperature from 1.1 to 39°C. The farm was located 212 m above sea level.

Before starting the experiment, proper approval for animal experimentation was obtained from the Institutional Animal Ethics Committee (IAEC). Murrah buffaloes (22) with 56 days before the expected date of parturition were selected from existing herd of Murrah buffaloes and divided randomly into two groups of 11 buffaloes each on the basis of their body weight (525.61±12.93 kg) and parity (2.5±0.44). All buffaloes were maintained under iso-managerial, individual and intensive feeding systems with housing in a well-ventilated concrete floor shed.

Buffaloes of both the groups were fed from 56 days prepartum to 95 days post-partum as per standard nutrient requirements (ICAR 2013) individually, using concentrate mixture (maize grain 32%, mustard cake 35%, wheat bran 30%, mineral mixture 2% and salt 1% during prepartum period, whereas cotton seed cake was included at the rate of 30% and mustard cake reduced up to 5% level in the concentrate mixture of lactating buffaloes) with equal amounts of available (20 to 30 kg/buffalo per day) green forage (to lactating buffaloes only) (berseem/mustard) with *ad lib.* wheat straw. Buffaloes of treatment group were additionally fed with a CMN supplement (from 56 days prepartum to 56 days post-partum) having Zn, Cu, Co, Cr, Se, vitamin E, vitamin A, and niacin) on weekly basis after properly mixing with small amount of concentrate mixture to ascertain its complete intake by animals and followed by offering the rest of the required amount of concentrate mixture to individual buffalo as per their nutrient requirements. The inclusion of CMN was withdrawn 56 days post-parturition with ultrasound examination until 95 days post-partum.

Reproductive performance of buffaloes was assessed using ultrasound examination at monthly interval post-parturition between days 25 to 35, 55 to 65 and 85 to 95. During the examination, the cyclicity status of the animals was diagnosed (to confirm the presence of corpus luteum) in addition to a number of large, medium and small follicles to ascertain the follicular dynamics.

Present address: [1]Lala Lajpat Rai University of Veterinary and Animal Science, Hisar, Haryana. [2]ICAR-Central Institute for Research on Buffaloes, Hisar, Haryana. ✉Corresponding author email: vishal.mudgal@icar.gov.in

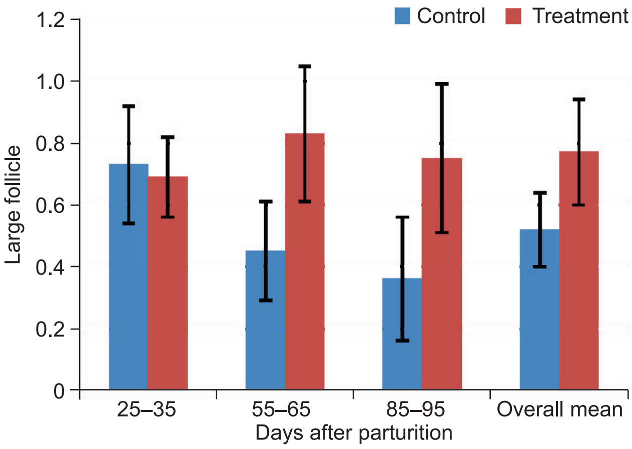

Fig. 1. Effect of weekly critical micro-nutrient supplementation on the number of the large follicles (>10 mm) in buffaloes.

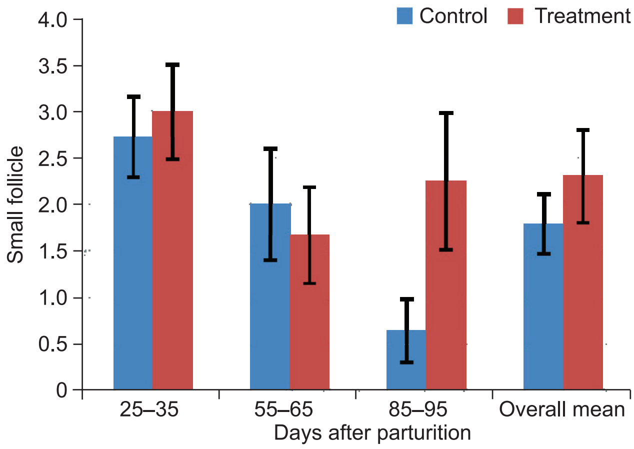

Fig. 3. Effect of weekly critical micro-nutrient supplementation on the number of the small follicles (1–4 mm) in buffaloes.

Data generated were analyzed statistically using the SPSS computer package (version 16) with a general linear model using the LSD test as Post Hoc test.

Dry matter intake remained comparable between two groups ranging from 1.46 to 1.56 kg/100 kg body weight during the prepartum period and 2.52 to 2.59 kg/100 kg body weight during post-partum period.

The mean of large follicles (≥10 mm) in buffaloes of two groups at the monthly interval after parturition with their overall mean values are presented in Fig. 1.

The values of large follicles in the treatment group remained comparable (P>0.05) to that of the control group throughout the study period, with overall mean values. Although, values were higher by about 85%, 108%, and 48% in the treatment group at 2nd and 3rd month with overall mean values, respectively as compared to control values. The mean of medium follicles (5–9 mm) in buffaloes of two groups at monthly intervals after parturition with their overall means values are presented in Fig. 2.

Values of medium follicles in two groups remained statistically comparable (P>0.05, but 63% and 52% high in buffaloes of the treatment group as compared to control) at day 25–35 and 55–65 but significantly high (P=0.036) values appeared at 85–95 d after parturition in the treatment group. The treatment group (1.51) had a tendency for higher (P=0.057) overall mean number of medium follicles compared with the control group (0.76) values. The mean of small follicles in buffaloes of two groups at monthly intervals after parturition with their overall means values are presented in Fig. 3.

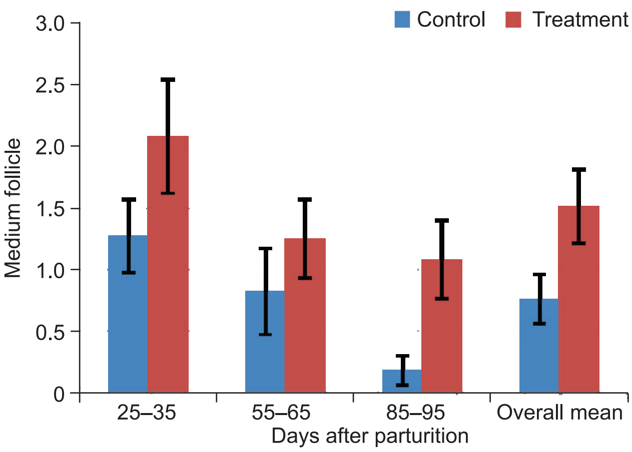

Fig. 2. Effect of weekly critical micro-nutrient supplementation on the number of the medium follicles (5–9 mm) in buffaloes.

The values of small follicles in the treatment group remained comparable (P>0.05) to that of the control group throughout the study period with their overall means (2.31 and 1.79 in the treatment and control groups, respectively).

Data regarding cyclicity (%) in buffaloes at the monthly interval is presented in Fig. 4. Data indicated a nonsignificant (P>0.05) difference in cyclicity (%) between the two groups throughout the study, but the overall effect was numerically better in the supplemented group.

CMN supplementation had no significant influence on a number of animals returns to cyclicity, but the effect was numerically apparent. More influence was visible on numbers of large (≥10 mm), medium (5–9 mm) and small follicle (1–4 mm). Several workers reported beneficial effects of different micronutrients on animal reproduction (Khan *et al.* 2014, Overton and Yasui 2014, Princewill *et al.* 2015). In contrast, Ullah *et al.* (2010) fed Ca, P, Zn and Mn either as per 100% of the recommendation level of National Research Council (NRC 2001) or 10% over NRC, but they did not report any effect on post-partum ovarian activity

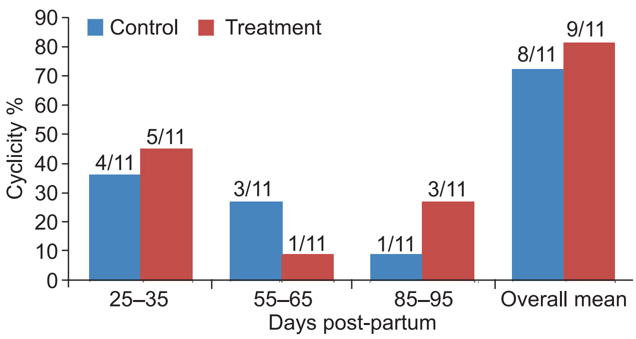

Fig. 4. Effect of weekly critical micro-nutrient supplementation on cyclicity % in buffaloes.

during different seasons in Nili Ravi buffaloes. Similarly, El-Brody *et al.* (2001) supplemented niacin in pregnant Egyptian buffaloes from 4 week prepartum to 12 week postpartum and reported a tendency to have a shorter interval from calving to detected estrous. They also required less (P<0.01) services per conception and had shorter (P<0.01) days open as compared with the control. Panda *et al.* (2006) when supplemented vitamin E in Murrah buffaloes during the periparturient period, reported a reduction in days open than their previous lactation performance. Deka *et al.* (2015) reported beneficial effects on the reproductive performance of buffaloes due to chromium supplementation in peri-parturient Murrah buffaloes (60 days to 150 days postpartum). Likewise, Soltan (2009) performed the study of Cr supplementation under heat stress in lactating dairy cows and showed a trend towards improving reproductive performance as indicated by the increased percentage of pregnant cows in the first 28 days of breeding. The season may be a big impact to affect, in the above study, supplemental micronutrients may be additionally helpful for the environmental stressful condition but in the present experiment, the study was conducted in more favorable conditions (winter season) and thus we were unable to get more stressful condition to affect the cyclicity performance adversely in the control group.

EL-Shahat and Abdel Monem (2011) supplemented vitamin E and selenium which led to a significant reduction in the meantime interval from supplementation to the first estrus as compared to control Egyptian Baladi ewes. Supplementation of vitamin E, vitamin A, and Zn reduced days open in crossbred dairy cows (Trojacanec *et al.* 2012). Similarly, De *et al.* (2014) studied the effect of vitamin E and mineral mixture supplementation (60 days prepartum to 60 days postpartum) and Khan *et al.* (2015) studied the effect of peripartum nutritional management (dietary protein and mineral mixture supplementation with injectable micro minerals at two months prior expected parturition and at day of parturition), reported an early initiation of cyclicity with better reproductive performance in supplemented than control groups.

The reproductive response may be due to a positive correlation between the progesterone concentration and level of β-carotene (Kalasariya *et al.* 2017) at the time of ovulation and during the luteal phase ($r = 0.33$; $P<0.05$ and $r = 0.55$; $P<0.01$, respectively). Ramos *et al.* (2012) and Qureshi *et al.* (2010) also reported significant reduction in days to first estrus and that may be due to the existence of a positive correlation between earlier resumption of ovarian cyclicity after parturition in cows with high serum β-carotene concentration before parturition and thus cows (Trojacanec *et al.* 2012) showed significant reduction in days to first estrus.

Critical micro-nutrients are involved in several cellular metabolic processes working either as the activator of enzymes or free radical detoxification as antioxidants. Supra-nutritional dose of certain antioxidant nutrients have beneficial effects (Ambily *et al.* 2019, Lashkari *et al.* 2018, McGrath *et al.* 2018, Mudgal *et al.* 2018, 2019; Vipin *et al.* 2020) on various blood parameters otherwise got exposed with oxidative stress during various normal physiological stages of life. Many of them act as components of hormones, thus directly regulating the endocrine activities. Due to their involvement in carbohydrate, protein and nucleic acid metabolism, they are also associated with the production of reproductive and other hormones, their state of imbalance may adversely affect embryonic development, post-partum recovery activities and overall fertility of animals (Kumar *et al.* 2011).

It could be interpretated that supplementation of critical micronutrients weekly during the periparturient period was helpful in improving the follicular dynamics in Murrah buffaloes.

SUMMARY

The study aimed to evaluate the effect of critical micronutrient (Zn, Cu, Co, Cr, Se, vitamin E, vitamin A, and niacin) supplementation at the weekly interval during the periparturient period (56 days prepartum till 56 days post-partum) on reproduction status of Murrah buffaloes. The experiment was performed on 22 healthy Murrah buffaloes after dividing them into two equal groups, representing control and treatment, respectively. A feeding schedule of both the groups was the same except that additional micronutrient supplementation was carried out once in a week in buffaloes of treatment group after proper mixing with concentrate mixture. Ultrasonography was performed once in a month for initial 90 days postpartum for assessment of cyclicity status of buffaloes. Treatment was unable to alter the cyclicity status of buffaloes for the initial 90 days but improved the numbers of large and medium-size follicles during the due course of the study. It may be concluded that weekly supplementation of critical micronutrients during the periparturient period is helpful in improving the follicular dynamics.

Compliance with Ethical Standards

Animal experiments followed the guidelines of the Institutional Animal Ethical Committee, constituted under the supervision of CPCSEA of India.

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