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in postpartum acyclic cattle mimics this short cycle and aids to induce cyclicity in a substantial proportion (Day, 2002). Satisfactory estrus induction and pregnancy rates following progesterone therapy have been reported in acyclic Sahiwal cattle (Singh et al., 2006). The effect of trace mineral profiles on efficacy of estrus induction protocols and conversely, behavior of mineral profiles during induction of cyclicity has not been well documented. Hence, present experiment evaluated such dynamics of the plasma Copper (Cu), Zinc (Zn), Manganese (Mn) and Iron (Fe) profiles in acyclic Sahiwal postpartum cows and post-pubertal heifers.

MATERIALS AND METHODS

Experimental animals and protocols

Healthy Sahiwal cows (2nd to 4th parity, body condition score= 2.5-4) with normal parturition and normal genitalia were subjected to gynecological examination at every 15 days interval up to three months postpartum.

Thereafter, a total of 25 cows from these confirmed for acyclicity (trans-rectal ultrasonography, twice at 10 days interval) were selected. Likewise, healthy post-pubertal Sahiwal heifers (n=25, ≥2½ years age, 250-350 kg average body weight) with anestrus condition (since attainment of sexual maturity) were selected. These animals were enrolled for induction of cyclicity using seven day intravaginal progesterone therapy (Fig. 1; protocol 1 in acyclic cows, n= 25; modified protocol 2 in heifers, n= 25).

The selected cows were identified with ear tags and not isolated from the herd. The controlled internal drug release device (CIDR, containing 1.38 g of natural progesterone, Inter AG. Hamilton, New Zealand) was applied as per standard procedure. Cows were observed for behavioral estrus aided by parading a teaser bull daily at 0600 and 1800 hours. Artificial insemination was done with good quality frozen semen. Pregnancy diagnosis at 60 days post-insemination was performed by trans-rectal ultrasonography.

Plasma trace mineral profiles

The concentration of trace minerals (Cu, Zn, Mn and Fe) in Jugular blood plasma samples was estimated by a double beam atomic absorption spectrophotometer using optimum instrumental settings and standard procedure. Standard curves were obtained by using different standards of known concentrations for each element. Each assay was checked intermittently by using different standards of that element.

Statistical Analysis

Analysis of the experimental data was carried out using analysis of variance (ANOVA) and Student’s t-test as described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Infertility in livestock arising from trace minerals deficiency is believed to be associated with enzymatic and endocrine dysfunctions along the hypothalamo-gonadal axis (Princewill et al., 2015). Our results indicated that plasma concentrations of copper, zinc and manganese influence the outcome of estrus induction protocols. In the
present experiment, the estrus induction and subsequent conception rates during the first induced estrus in postpartum cows were 68% and 41.2%, respectively. The corresponding values in post-pubertal heifers were 64% and 50%, respectively (Fig. 2).

Fig. 2: Fertility response of CIDR based protocols in anestrus Sahiwal cows and heifers

The plasma concentrations of Cu, Zn, Mn and Fe in treated cows and heifers are presented in Fig. 3 and 4. Significant variation (P<0.05) in plasma concentrations of Cu and Zn was observed both in cows and heifers during the course of CIDR application. There was an increasing trend of Cu concentrations in cows as well as heifers. Contrarily, the manganese concentrations varied significantly (P<0.05) only in cows and not in heifers.

Fig. 3: Plasma concentrations (μg/ml) of trace minerals (Mean±SE) during the course of estrus induction in postpartum Sahiwal cows

However, iron concentrations remained uniform during the course of treatment both in cows and heifers. The lower Cu concentration in anestrus animals has been attributed to prevailing lower estrogen profile (Rajkumar et al., 2006). Likewise, increased plasma concentration of Cu (with no effect on Zn) was reported following administration of estrogen (Sato and Henkin, 1973). Hence, the variation in mineral concentrations recorded in present experiment may perhaps be due to endocrine changes following activation of reproductive axis during estrus induction. However, further investigations are warranted to establish such changes in mineral profiles.

Fig. 4: Plasma concentrations (μg/ml) of trace minerals (Mean±SE) during the course of estrus induction in post pubertal Sahiwal heifers

Pre-treatment concentrations of trace minerals were also compared with respect to the outcome of treatment (estrus induced vs. not induced). No significant difference (P>0.05) was recorded for iron concentration vis-à-vis estrus induction response (Fig. 5 and 6). In contrast, the mean pre-treatment concentrations of copper, zinc and manganese were significantly (P<0.05) higher both in cows and heifers in which estrus induction was successful as compared to those in which estrus induction failed to occur. This indicated that the success of estrus induction protocols also depends on circulatory trace mineral status especially copper, zinc and manganese. The role of trace minerals in maintaining optimum fertility has been demonstrated in various studies through its involvement in GnRH, FSH, LH and estrogen activity (Michaluk and Kochman, 2007). Suboptimal ovarian activity, delayed or depressed estrus (Mudgal et al., 2018), and delayed puberty were reported in cattle and buffaloes grazing on pastures low in Cu (Kumar et al., 2003). On the contrary,
Das et al. (2009) recorded no significant difference in serum copper profiles among crossbred cattle with various ovulatory disturbances (delayed ovulation, anovulation) and normal ovulation. Copper is essential for the activity of superoxide dismutase enzyme, the deficiency of which has been associated with defects in ovarian folliculogenesis (Lei et al., 2015).

Zinc (Zn) also plays an essential component of enzymes in different biochemical pathways and thus plays a pivotal role in reproduction. Delayed puberty in heifers (Ahmed et al., 2002), lower conception rates and failure of implantation in Zn deficient cows have been recorded (Verma and Kumar, 2018), while its supplementation increased calving rates in heifers (Patel et al., 2017). Zinc deficiency leads to suppression of hypothalamo-pituitary-gonadal axis and is directly involved in activity of steroid receptors owing to its presence in the form of ‘Zn fingers’ (Kluska et al., 2018).

Our study recorded that manganese concentration also influenced the outcome of estrus induction protocols, and the failure of estrus induction may be due to marginal manganese deficient state. Poor follicular development with delayed ovulation, reduced intensity of estrus and reduced conception rates has been associated with Mn deficiency (Kumar et al., 2011). Manganese supplementation has proven to be effective in shortening the postpartum anestrus period and increasing conception rates in dairy animals (Ullah et al., 2010). The lower serum Mn concentration was reported in delayed ovulating and an ovulating compared to the normal ovulating heifers (Das et al., 2009). Although the precise mechanism pertaining to the involvement of manganese in reproduction is unknown, its deficiency alters the synthesis of estrogen and progesterone in the females, possibly through the inhibition of cholesterol and cholesterol precursor synthesis (Pradhan and Nakagoshi, 2008).

Iron concentrations in the present experiment were within the physiological range. There was no significant variation during the course of treatment and also between responsive versus unresponsive animals, indicating perhaps an optimal supply of this micronutrient. In contrast, Lower level of serum iron plays a significant role in causing failure of conception and embryonic death (Modi et al., 2013). Deficiency of Fe in dairy cows rarely occurs, except in cases of some parasitic and infectious diseases as most of the conventional diets contain sufficient amounts of Fe (Theil, 2004).

The results indicate that Cu, Zn and Mn exert influence...
on reproductive synergy/performance and their deficiency may influence the outcome of estrus induction protocols in acyclic cattle. Hence it becomes essential to optimize circulatory profiles of trace minerals before induction of cyclicity or synchronization.

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