EFFECT OF HORMONE THERAPY ON FERTILITY AND PLASMA MINERALS PROFILE IN REPEAT BREEDING GIR COWS

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ABSTRACT

This study was carried out on 24 repeat breeding cows of Gir breed divided at random in to four groups, 6 animals in each. Animals of Group I were treated with single i/m injection of 0.02 mg buserelin acetate and Group II with single i/m injection of 1500 IU hCG immediately after natural breeding, while animals of Group III were administered with 500 mg of hydroxy-progesterone caproate on day 6 of natural breeding and those of Group IV bred without any treatment served as control. Blood plasma samples were collected on day 0 (oestrus) and then at weekly interval up to 6 weeks post-breeding/ treatment and were stored at -20°C. The first service conception rates obtained following 3 treatments were 50.00, 83.33 and 50.00 %, respectively, with an overall mean of 61.11 %. These were significantly higher (P<0.01) than that (16.66 %) obtained in untreated control group. The overall mean values of plasma calcium, inorganic phosphorus, Ca:P ratio and magnesium were 11.918±0.159 mg/dl, 8.193± 0.123 mg/dl, 1.516±0.032 and 1.818±0.028 mEq/L without significant differences between weeks or between conceived and non-conceived groups, except magnesium, the level (mEq/L) of which in GnRH treated group (2.341±0.047) was significantly (P<0.05) higher than in hCG and Progesterone treated groups (1.731 ±0.023 and 1.620±0.021). The overall mean values of trace elements, viz., zinc, iron, copper and manganese were 1.549±0.008, 2.119±0.023, 0.779±0.005 and 0.090±0.001 ppm, respectively. The values of zinc and copper were significantly (P<0.05) higher in all three treated groups as compared to control group. The values were significantly higher (P<0.01) in conceived group as compared to non-conceived group for all four elements, and varied significantly between weeks for copper and manganese. Overall, the hormonal therapy, especially hCG, was found highly effective in solving repeat breeding problem in Gir cows, though it did not influence the plasma mineral profile.

KEY WORDS: Gir cows, repeat breeding, hormone therapy, plasma mineral profile.

INTRODUCTION

Repeat breeding syndrome in dairy animals is a challenging and frustrating problem caused by certain known and unknown reasons and many hormonal and non-hormonal drugs have been tried by the scientists and veterinarians for its amelioration with varying success. Micro-minerals have a great impact on animal's reproductive physiology and its imbalance causes various problems leading to lowered reproductive efficiency and resultant monetary loss to the dairy industry. Often correcting an imbalance in mineral levels can solve a nagging problem by improving reproductive performance and health with little additional cost. As the terrain and the agro-climatic areas of Indian sub-continent are quite diverse, one therapeutic treatment of a region may not be suitable for other regions (Kumar et al., 2011). Hence, this study was planned to evaluate the efficacy of different hormone therapies at breeding on conception rate and weekly plasma profile of macro- and micro-minerals in repeat breeding cows.

MATERIALS AND METHODS

The study was carried out at Gaumandir Gaushala, Vadodara on 24 repeat breeding Gir cows beyond 6 months postpartum during the year 2011-12. The cows were selected on the basis of past records and through gynaeco-clinical examinations per rectum. Animals were managed under loose housing system and were fed green fodder, hay and concentrate. Heat detection was carried out visually twice daily and the females in oestrus were bred by a normal fertile bull. Pregnancy was confirmed by rectal palpation 60 days post-breeding. All the animals were administered with i/m injection of oxytetracyclin dihydrate long acting (Pfizer) @ 8-10 mg/kg body weight to check genital infection, if any, and were dewormed with 3 gm Panacur bolus before starting the hormonal treatment. The cows were equally and randomly divided in to four groups, each of six animals, for the therapeutic management as well as for the evaluation of weekly plasma macro-micro minerals profile post-treatment as under.

Group 1: Single i/m injection of 0.02 mg buserelin acetate (GnRH analogue; 5 ml Receptal, Intervet India Ltd.) immediately after breeding.

Group 2: Single i/m injection of 1500 IU hCG (Chorulon, Intervet India Ltd.) immediately after breeding.

Group 3: Single i/m injection of 500 mg hydroxyl progesterone caproate (Proluton depot, Zydus Cadila India) on 6th day of breeding.

Group 4: Six repeat breeder animals bred without any treatment served as control group.

All the cows were followed for conception or return to oestrus with collection of heparinized jugular blood samples on day 0 (oestrus) and then at weekly interval up to 6 weeks post-breeding, except in group 3 where a sample was also collected on day 6 post-breeding (just before treatment) and plasma samples were stored at -20°C until analyzed. Plasma calcium, inorganic phosphorus and magnesium concentrations were estimated using standard procedures and assay kits procured from Crest Biosystems, Goa, with the help of Chemistry Analyser (Mindray, BS 120). For the assay of micro-minerals profile, plasma samples (1 ml each) were wet digested with 5 ml volume of di-acid mixture (Perchloric acid: Nitric acid, 1:4) on a hot plate (Krishna and Ranjhan, 1980). The clear transparent residues were diluted in triple glass distilled water and final volume was made to 5 ml. These aliquots were then used for estimation of trace elements, viz., zinc, iron, copper and manganese on an Atomic Absorption Spectrophotometer (Model-3110, Perkin Elmer).

The data on weekly minerals profile were analyzed using two factors factorial CRD (4 groups x 6 weeks x 6 animals) and those of conceived and non-conceived groups by using simple CRD and Duncan's NMRT and Student's 't' test.

RESULTS AND DISCUSSION

Fertility Response:

The conception rates following single i/m use of 0.02 mg GnRH and 1500 IU hCG soon after breeding and 500 mg hydroxy-progesterone on day 6 of breeding in repeat breeding cows were 50.00 (3/6), 83.33 (5/6) and 50.00 (3/6) %, respectively. The overall pooled conception rate of 3 groups was significantly ($P<0.01$) higher than in untreated control group (61.11 vs 16.66 %). These results with gonadotrophins compared well with many of the previous reports (Anjum et al., 2009; Dodamani et al., 2010). Gonzalez et al. (1999) reported that GnRH-agonist had a luteotrophic effect that delayed regression of corpus luteum and maintained high progesterone concentration. The present level of enhanced conception rate with hCG treatment is higher than that reported by Ambrose et al. (2002). The improved conception rate obtained with hCG might be due to its beneficial effect towards regulating the time of ovulation as well as luteinizing effect on mature follicle. The findings with progesterone treatment corroborated with the report of Das et al. (2002) and Singh and Nanda (2007). Embryonic loss before 15 days of gestation is usually preceded by and may be caused by luteal regression. The results in general indicated beneficial role of hormone therapy, particularly hCG, towards improving conception rate in repeat breeding Gir cows.

Plasma Macro-Minerals:

The overall pooled mean plasma concentrations of calcium, inorganic phosphorus, Ca:P ratio and magnesium found in cows were 11.918 ± 0.159 mg/dl, 8.193 ± 0.123 mg/dl, 1.516 ± 0.032 and 1.818 ± 0.028 mEq/L, respectively (Table 1). The levels of only phosphorus and magnesium varied significantly ($P<0.05$) between weeks post-treatment. The hormonal treatment, viz., GnRH, HCG and progesterone, however, did not influence their profile over 6 weeks period studied (Table 2). Present results on calcium approximated with the finding of Chaurasia et al. (2010) in buffaloes. The pooled mean plasma calcium levels were higher in hCG treated group and lower in GnRH and progesterone treated groups as compared to untreated control, although the differences were not significant. These findings contradict with the observations of Sheshappa et al. (2002). Average plasma calcium levels were found to be the lowest on day '0' i.e, on the day of oestrus in almost all groups, which might be due to high level of oestrogen, which stimulates calcium uptake in the bones. Moreover, the inorganic phosphorus levels were significantly ($P<0.05$) lower and Ca:P ratio higher on day 0 and 1st week, compared to other weeks. These levels are in normal physiological range.

The mean plasma calcium and phosphorus levels between conceived and non-conceived groups did not vary significantly, though the variation between weeks was significant for phosphorus in conceived group (Table 2). This agreed with Ahlawat (2003). Dhoble and Gupta (1986) opined that calcium appears to have indirect relation with reproduction in cattle. Ruginuso et al. (2011) reported that the mean serum calcium value in repeat breeding cows was lower than that obtained in the control group. The role of calcium in sensitizing the tubular genitalia for the action of hormone is well established. Hurley et al. (1980) suggested that the fertility of animals tended to be reduced if the inorganic phosphorus level falls, while increased phosphorus level was related to the improvement of ovarian activity. Das et al. (2009) and Chaurasia et al. (2010) observed that serum phosphorus concentration was significantly higher ($P<0.01$) in normal cyclic than repeat breeding cows. According to Herrick (1977) the disturbed Ca:P ratio has a blocking action on the pituitary and consequently on the ovary and it may prolong the interval to first ovulation.

The mean magnesium concentration was significantly higher in GnRH and hCG treated groups as compared to progesterone treated and control group, though it did not vary between conceived and non-conceived groups. Chaurasia et al. (2010) also reported insignificant differences in concentrations of magnesium among normal cyclic, repeat breeder and anoestrus buffaloes. The weekly average plasma magnesium levels in non-conceived groups varied significantly as noted by Ahlawat (2003). Kalita and Sarmah (2006) did not find significant variation in magnesium levels of normal cyclic and repeat breeding cows. Patel (2004), however, reported significantly lower mean plasma magnesium in conceived than non-conceived HF cows. Das et al. (2009) observed that the concentration of magnesium was found to be significantly lower in the animals with anovulation compared to those with normal and delayed ovulation. Magnesium requirement seems to be more pronounced at the time of occurrence of high energy requiring processes because association of magnesium is well known as a co-factor in all the ATP requiring enzymatic processes in overall general metabolism, nevertheless reproduction is the most dominating process in a biological system.

Table 1. Overall mean (± SE) plasma concentrations of macro- and micro-minerals in repeat breeding Gir cows of different treatment groups and in conceiving and non-conceiving groups

Plasma minerals	Treatment Groups				Overall Pooled (n=24)	Reproductive Status	
	GnRH (n=6)	hCG (n=6)	Progesterone (n=6)	Control (n=6)		Conceived (n=12)	Non-C (n=12)
Calcium (mg/dl)	11.870 ± 0.237	12.259 ± 0.313	11.476 ± 0.362	12.066 ± 0.343	11.918 ± 0.159	12.185 ± 0.228	11.650 ± 0.218
Phosphorus (mg/dl)	8.135 ± 0.214	8.474 ± 0.234	8.294 ± 0.287	7.871 ± 0.250	8.193 ± 0.123	8.363 ± 0.173	8.024 ± 0.176
Ca:P ratio	1.512 ± 0.058	1.498 ± 0.061	1.436 ± 0.057	1.616 ± 0.790	1.516 ± 0.032	1.518 ± 0.046	1.514 ± 0.045
Magnesium (mEq/L)	2.341 ± 0.047^{z}	1.731 ±0.023^{y}	1.620 ± 0.021^{x}	1.581 ± 0.029^{x}	1.818 ± 0.028	1.819 ± 0.033	1.817 ± 0.046
Zinc (ppm)	1.570 ± 0.015^{y}	1.561 ± 0.015^{y}	1.591 ± 0.015^{y}	1.473 ± 0.018^{x}	1.549 ± 0.008	1.587 ± 0.012	1.511 ± 0.011^{**}
Iron (ppm)	2.255 ± 0.041^{y}	2.167 ± 0.030^{y}	2.032 ± 0.038^{x}	2.023 ± 0.060^{x}	2.119 ± 0.023	2.238 ± 0.018	2.001 ± 0.038^{**}
Copper (ppm)	0.781 ± 0.011^{y}	0.818 ± 0.012^{z}	0.781 ± 0.012^{y}	0.737 ± 0.007^{x}	0.779 ± 0.005	0.834 ± 0.007	0.724 ± 0.002^{**}
Manganese (ppm)	0.091 ± 0.002	0.091 ± 0.002	0.089 ± 0.001	0.090 ± 0.001	0.090 ± 0.001	0.094 ± 0.001	0.087± 0.001^{**}

n = Number of animals; ** Highly significant (P<0.01) between conceived and non-conceived groups.

Means of treatment groups bearing uncommon superscripts differ significantly (P<0.05).

Plasma Micro-Minerals Profile:

The overall pooled mean plasma levels of zinc, iron, copper and manganese were 1.549±0.008, 2.119±0.023, 0.779±0.005 and 0.090±0.001 ppm, respectively (Table 1). The values of zinc and copper were significantly (P<0.05) higher in all three treated groups as compared to control group. Similarly, the values of all four elements were significantly higher (P<0.01) in conceived than non-conceived group, and they varied significantly between weeks for copper and manganese (Table 3). These observations agreed well with the findings of Kalita and Sarmah (2006). The findings indicated that low level of zinc in the blood may be related to delayed postpartum conception. Das et al. (2009) observed that serum concentrations of zinc and manganese, but not iron, were significantly (P<0.01) higher among the animals with normal ovulation than with delayed ovulation and anovulation. Zinc is closely related with Vit-A functioning in the body tissues including the gonads. Adequate zinc levels are vital for repair of the uterine lining following calving, return to normal oestrous cycles and maintenance of the uterine lining necessary for implantation of embryos. Zinc is an important component in most enzyme systems, RNA and DNA and in regulating hormones related to production and reproduction. Zinc deficiency is documented as being responsible for sterility due to defects in prostaglandin metabolism.

Table 2. Weekly mean (± SE) plasma profile of macro-minerals in conceiving (C) and non-conceiving (NC) repeat breeding Gir cows following various hormonal treatments at breeding

[redacted]

Means of treatment groups bearing uncommon superscripts differ significantly (P<0.05).

Table 3. Weekly mean (± SE) plasma trace minerals (ppm) in conceiving (C) and non-conceiving (NC) repeat breeding Gir cows following various hormonal treatments at breeding

[redacted]

Means of treatment groups bearing uncommon superscripts differ significantly ($P<0.05$).

Mean plasma iron concentration in GnRH and hCG treated group was significantly ($P<0.05$) higher than that of control group, and also in conceived than in non-conceived cows, thus agreed with report of Kavani et al. (2005), indicating beneficial role of iron in fertility. Further, significant ($P<0.01$) difference was observed between the groups in 3rd, 4th and 6th weeks. Moreover, Iron is involved as a co-enzyme in number of enzymatic reactions. The lower level of iron could possibly results in improper tissue oxygenation to the uterus resulting in impaired nutrition to the uterus (Reddy and Reddy, 1988). Iron is required for the synthesis of myoglobin as well as many enzymes and cytochrome enzymes of electron transport chain. A deficient animal becomes repeat breeder and requires increased number of inseminations per conception and occasionally may abort.

The mean plasma concentration of copper was significantly ($P<0.05$) higher in hCG group than GnRH and Progesterone group, and in conceived than in non-conceived group, but no particular trend was observed between weeks for any of these groups (Table 3). Cummins and Harris (1984) reported that there is adverse effect of copper on fertility. Ahlawat (2003) recorded high level of copper in non-conceived than the conceived cows. Wiener and Sales (1976), however, documented that copper level in the blood alone may not be the true index of reproductive dysfunction. It is theorized that reproductive efficiency is reduced due to alterations of enzyme systems caused by low copper levels. Copper deficiency can result in delayed oestrus, decreased conception rates, infertility and early embryonic death.

The overall mean manganese concentration was significantly ($P<0.01$) higher in conceived than in non-conceived group but without significant differences among weeks in either of the groups. Significant difference ($P<0.05$) was also observed between groups on 4th week post-breeding (Table 3). However, Ahlawat (2003) and Patel (2004) did not find significant difference in plasma manganese levels between conceived and non-conceived cows. Granulose cells of the ovary require manganese for the follicular development, thus its deficiency might lead to degeneration of granulose cells. Manganese deficiency may directly impair ovarian activities or indirectly through breakdown of the hypothalamo-pituitary feedback mechanism. Manganese is an activator of a number of enzymes both of somatic cells and gonads and thus its deficiency leads to the signs of disturbed or depressed oestrus. Manganese requirement for reproduction is enhanced if the dietary content of calcium and phosphorus are higher, depicting interrelationship of Ca-P-Mn (Little John and Lewis, 1960).

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