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Bio-stimulatory effects of bull urine (BU), PRID and GnRH on hormonal profile and association with blood minerals in pubertal anestrus Sahiwal heifers

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ABSTRACT

The present study was designed to assess the bio-stimulatory effect of bull urine (BU) exposure, administration of progesterone impregnated intravaginal device (PRID) in combination with $PGF_2\alpha$ and gonadotropin releasing hormone (PRID-PG-GnRH), and GnRH in combination with $PGF_2\alpha$ (GnRH-PG-GnRH) on alteration of hormonal profile in correlation with induction of estrus in pubertal anestrus Sahiwal heifers. Pubertal anestrus Sahiwal heifers (32) were randomly divided into 4 groups, viz. group 1 (8; BU), group 2 (8; PRID-PG-GnRH), group 3 (8; GnRH-PG-GnRH) and group 4 (8; Control). Treatment with PRID-PG-GnRH and GnRH-PG-GnRH significantly increased the serum progesterone and estrogen concentration whereas, in BU and untreated control heifers, the progesterone concentration remained at the basal level during 30 days observation period. However, a meager increase of serum estrogen concentration was observed from day 9 and 11 in BU treated heifers. The association of certain macro and micro minerals level in blood during pre and post treatment period was studied to correlate the treatment response. Significant difference was observed in mean serum calcium (mg/dl) and iron (ppm) levels in pre and post BU exposed, PRID-PG-GnRH and GnRH-PG-GnRH treated heifers compared to the control. No significant difference in mean serum phosphorus (mg/dl), copper (ppm) and cobalt (ppm) level was observed in all the groups. Significant difference was observed in mean serum magnesium (mg/dl) and zinc levels in pre and post PRID-PG-GnRH and GnRH-PG-GnRH treated heifers as compared to BU exposed and control heifers. Thus, it can be concluded that the treatment with PRID-PG-GnRH and GnRH-PG-GnRH had more pronounced effect on induction of estrus as reflected by increased level of serum estradiol on day 9 and progesterone level on 10^{th} day post AI than the bull urine treated and control heifers and subsequent increase in serum calcium, magnesium, iron, and zinc levels during post treatment period in pubertal anestrus Sahiwal heifers.

Key words: Bull urine, Hormone, Minerals, Pubertal anestrus, Sahiwal heifers

Reproductive efficiency is considered to be one of the key components in profitable dairy farming. Delayed puberty or prolongation in the breeding rhythm results in progressive economic loss due to extended dry period, reduced lifetime calving and milk yield per lactation. Earliest possible age at first service has a positive effect on genetic progress to optimize lifetime productivity (Day and Grum 2005). Physiological reproduction and production performance is constantly under the influence of interrelationship between nutrition and endocrine. Insufficient production and release of pituitary gonadotropins impairs normal ovarian follicular responsiveness (Ozyurtlu *et al*. 2009). The normal reproductive processes in animal depends on different nutrients including proteins, vitamins and minerals, their deficiency often causes hypothalamic-pituitary mediated ovarian dysfunction resulting in extended anestrous period in cows (Jayachandran *et al.* 2013). Different metabolic energy and mineral alterations lead to negative impacts on fertility. Lower circulatory minerals result in impaired reproductive functions leading to cessation of cyclic activity (Niazi *et al*. 2003). Deficiency of a single or multiple minerals may cause infertility, poor conception and anestrus (Hidiroglou 1979). Attempts have been made on the regulation and control of bovine reproduction by the use of various reproductive hormones (Patterson *et al.* 2003), drugs, chemicals, biological agents and management practices (Hopkins 1986) appear to be cost effective. Application of hormones has good therapeutic value to enhance the reproductive efficacy in infertile animals with good nutritional status (Ghuman *et al.* 2009, Savalia *et al.* 2014). The bio-stimulatory effect of bull urine was found effective for estrus induction in delayed pubertal and

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postpartum anestrus cows (Hornbuckle *et al.* 1995, Ahmed *et al*. 2016). The bio-stimulation effect is mediated through pheromones to influence the recipient's endocrine milieu of reproductive system and exhibits physiological and behavioral changes. Effects of bio-stimulation on reproductive hormone and association with the mineral profile in pubertal anestrus Sahiwal heifers have not been investigated. Therefore, this study was designed to investigate the effect of BU exposure, PRID-PG-GnRH and GnRH-PG-GnRH protocols on estradiol and progesterone profile and the association of blood mineral status of pubertal anestrus Sahiwal heifers.

MATERIALS AND METHODS

The experiment was conducted with pubertal anestrus Sahiwal heifers (32) at an average age of 35 months, maintained at the Bull Mother Experimental Farm, College of Veterinary Science and Animal Husbandry, Anjora, Durg. The experimental heifers were randomly divided into 4 groups, viz. group 1 (8; bull urine), group 2 (8; PRID-PG-GnRH), group 3 (8; GnRH-PG-GnRH) and group 4 (8; control) and managed in iso-managerial condition. The animals of group 1 were sprayed with bull urine (3 ml/animal/day) on the oronasal part of the heifers daily until onset of estrus or maximum of 30 days. The bull urine was collected on alternate days in a sterilized glass beaker from different Sahiwal bulls (4 to 5 years age) maintained at Central Semen Station, Anjora, Durg (Chhattisgarh). Immediately after collection, the urine was filtered through Millipore (0.45 μm) membrane filter and stored in different aliquots at 4°C till use. In group 2, the progesterone-releasing intravaginal device (PRID) was inserted in to the vagina and left *in situ* for 7 days. On day 7, PRID was removed and 0.5 mg cloprostenol ($PGF_2\alpha$ analogue) was administered intramuscularly. In group 3, 10 μg buserelin acetate (GnRH synthetic analogue) and 0.5 mg cloprostenol was administered intramuscularly on day 0 and 7, respectively. All the treated animals were observed visually twice daily at morning (7 am) and evening (4 pm) for estrus signs after $PGF_2\alpha$ injection. The heifers in estrus were confirmed by per rectal examination; 48 h later (day 9), all animals were inseminated (fixed timed artificial insemination, FTAI) with frozen-thawed semen from superior-proven Sahiwal bull and 10 μg Buserelin acetate was administered intramuscularly. The heifers in control group were not given any treatment except regular estrus detection like in treatment groups.

The blood samples were collected from all experimental animals in treatment and control groups in vaccutainer tubes by jugular venipuncture using 20G sterile disposable needle on day 0, 3, 7, and 22nd day (10th day post AI) in group 2 and 3. Similarly, blood samples were collected on day 9, 12, 15, 18, 22, 26, 30 in group 1 and 4 following initiation of treatment. The serum was harvested from blood samples after centrifugation at 3,000 rpm for 15 min and was stored in different aliquots at –20°C till analysis of hormone and minerals. Progesterone and estradiol 17 β were estimated using commercial kits (RIA, Immunotech, France) and the trace elements (magnesium, copper, cobalt, iron and zinc) were estimated in an atomic absorption spectrophotometer (Model AAS 4141), as described by Oser (1979). The blood serum of calcium and phosphorus were analyzed by semi auto-analyzer (Systronics India Ltd.) using coral clinical systems diagnostic kits as per specification of instrument manual. All the data were analyzed for one way analysis of variance (ANOVA) with the help of statistical package (SPSS).

RESULTS AND DISCUSSION

Bio-stimulatory effects of bull urine (BU), PRID-PG-GnRH and GnRH-PG-GnRH on progesterone (ng/ml) and estrogen (pg/ml) hormone in pubertal anestrus Sahiwal heifers are presented in Table 1. There was no significant effect of bull urine on serum progesterone from day 0 to day 30. However, there was significant difference in the serum estrogen from day 9 and 11. There was significant effect of PRID-PG-GnRH and GnRH-PG-GnRH treated heifers on serum progesterone ($P<0.01$) and estrogen

Table 1. Least square mean (±SE) of serum progesterone (P4; ng/ml) and estrogen level (E2; pg/ml) in pubertal anestrus Sahiwal heifers

Days	P4 hormone				E2 hormone			
	Group 1 (BU)	Group 2 (PRID)	Group 3 (GnRH)	Group 4 (Control)	Group 1 (BU)	Group 2 (PRID)	Group 3 (GnRH)	Group 4 (Control)
0	0.21 ± 0.09^{aA}	0.33 ± 0.18^{aA}	0.29 ± 0.15^{aA}	0.19 ± 0.08^{aA}	3.60 ± 0.14^{aA}	3.90 ± 0.17^{aA}	3.61 ± 0.22^{aA}	3.45 ± 0.41^{aA}
3	0.24 ± 0.05^{aA}	0.71 ± 0.14^{aA}	0.65 ± 0.27^{aA}	0.20 ± 0.04^{aA}	3.57 ± 0.39^{aA}	4.85 ± 0.24^{aA}	5.60 ± 0.34^{aA}	3.37 ± 0.36^{aA}
7	0.27 ± 0.10^{aA}	5.21 ± 0.32^{bB}	4.90 ± 0.51^{bB}	0.19 ± 0.08^{aA}	3.54 ± 0.42^{aA}	4.89 ± 0.29^{aA}	5.81 ± 0.19^{aA}	3.35 ± 0.15^{aA}
9	0.31 ± 0.06^{aA}	0.91 ± 0.21^{aA}	0.53 ± 0.19^{aA}	0.21 ± 0.04^{aA}	4.55 ± 0.13^{aA}	9.39 ± 0.15^{bB}	10.36 ± 0.66^{bB}	4.23 ± 0.23^{aA}
11	0.35 ± 0.11^{aA}	-	-	0.27 ± 0.07^{aA}	4.43 ± 0.28^{aA}	-	-	4.05 ± 0.18^{aA}
15	0.39 ± 0.08^{aA}	-	-	0.20 ± 0.03^{aA}	4.25 ± 0.63^{aA}	-	-	4.12 ± 0.14^{aA}
18	0.44 ± 0.13^{aA}	-	-	0.26 ± 0.12^{aA}	4.07 ± 0.47^{aA}	-	-	4.20 ± 0.29^{aA}
22	0.54 ± 0.18^{aA}	4.32 ± 0.29^{bB}	3.93 ± 0.37^{bB}	0.17 ± 0.09^{aA}	3.65 ± 0.58^{aA}	4.83 ± 0.49^{aA}	5.83 ± 0.43^{aA}	3.79 ± 0.73^{aA}
26	0.48 ± 0.15^{aA}	-	-	0.26 ± 0.05^{aA}	3.22 ± 0.43^{aA}	-	-	3.28 ± 0.91^{aA}
30	0.43 ± 0.13^{aA}	-	-	0.23 ± 0.08^{aA}	3.41 ± 0.19^{aA}	-	-	3.52 ± 0.52^{aA}

Values with different superscript in same row (small letters) and same column (capital letters) for same hormone differ significantly.

Table 2. Least square mean (±SE) of blood serum macro and micro minerals in pubertal anestrus Sahiwal heifers during pre and post treatment period

Mineral	Schedule	Group 1 (BU)	Group 2 (PRID)	Group 3 (GnRH)	Group 4 (Control)
Calcium (mg/dl)	Pre-treatment	9.25±0.28[a]	8.87±0.16 [a]	9.51±0.36 [a]	9.32±0.29
	Post-treatment	10.83±0.36[b]	10.07±0.23 [b]	10.35±0.19 [b]	9.13±0.14
Phosphorus (mg/dl)	Pre-treatment	3.72±0.25	3.61±0.36	3.20±0.82	3.42±0.31
	Post-treatment	4.05±0.15	3.92±0.19	3.48±0.21	3.72±0.14
Magnesium (mg/dl)	Pre-treatment	2.13±0.12	2.42±0.19 [a]	2.32±0.34 [a]	2.04±0.09
	Post-treatment	2.29±0.41	2.70±0.24 [b]	2.55±0.16 [b]	2.15±0.14
Copper (ppm)	Pre-treatment	0.76±0.07	0.74±0.15	0.76±0.06	0.71±0.27
	Post-treatment	0.85±0.14	0.88±0.21	0.81±0.12	0.79±0.17
Cobalt (ppm)	Pre-treatment	1.44±0.08	1.42±0.11	1.59±0.15	1.32±0.17
	Post-treatment	1.47±0.07	1.51±0.19	1.64±0.13	1.35±0.21
Iron (ppm)	Pre-treatment	4.27±0.08 [a]	5.22 ± 0.32[a]	5.51±0.14 [a]	3.94±0.21
	Post-treatment	5.96±0.13 [b]	6.86±0.17 [b]	7.16±0.19 [b]	4.17±0.15
Zinc (ppm)	Pre-treatment	1.41±0.05	1.36±0.14 [a]	1.28±0.04 [a]	1.23±0.13
	Post-treatment	1.52±0.09	1.63±0.11 [b]	1.66±0.18 [b]	1.27±0.17

Values with different superscript (a,b) in the same column between pre and post treatment differ significantly (P<0.05).

(P<0.05) concentration. Our findings with bull urine are in agreement with the reports of Patra *et al.* (2010) in crossbred heifers. Ahmad *et al.* (2010) reported similar findings in postpartum crossbred cows; to the contrary, Hornbuckle *et al.* (1995) reported higher concentration of progesterone in beef cows exposed to bull. Significant difference in mean serum progesterone and estrogen concentration was observed in anestrus Sahiwal heifers treated with PRID-PG-GnRH and GnRH-PG-GnRH and corroborate with the reports of Ergene (2012). The study supports the findings of Ahmed *et al*. (2016) who reported a significantly lesser estrus induction and follicular growth pattern following BU exposure in pubertal anestrus Sahiwal heifers than PRID-PG-GnRH and GnRH-PG-GnRH treatment.

Pre and post-treatment mean serum mineral levels among the 4 groups are presented in Table 2. Significant difference (P<0.05) was observed in mean serum calcium (mg/dl) and iron (ppm) levels in pre and post BU exposed, PRID-PG-GnRH and GnRH-PG-GnRH treated heifers, whereas, no significant difference was observed in control heifers. Our findings on blood calcium level are in agreement with the reports of Shrivastava and Kadu (1995), Kalita *et al.* (1999) and Singh *et al.* (2005) who also observed higher serum calcium concentration in normal cyclic heifers than delayed pubertal anestrus heifers. Blood calcium level indirectly influences the treatment response in pubertal anestrus heifers and appears to affect the reproductive efficiency (Virmani *et al.* 2011). Dhoble and Gupta (1986) also found higher calcium level in cyclic buffaloes as compared to acyclic animals. No significant difference in mean serum phosphorus (mg/dl), copper (ppm) and cobalt (ppm) level in pre and post BU exposed, PRID-PG-GnRH and GnRH-PG-GnRH treated and control heifers. Similar phosphorus findings were reported by Sahu (2002) in normal cyclic (4.74±0.48 mg/dl) and anestrus (4.44±0.39) heifers. The phosphorus levels of present study are in agreement with the findings of Agarwal *et al.* (1985) and Sivaiah *et al.* (1986) in anestrus and cyclic cows. However, Virmani *et al.* (2011) and Dhoble and Gupta (1986) observed lower phosphorus level in anestrus buffaloes as compared to the normal cycling buffaloes. Dutta *et al.* (2001) reported higher serum copper level in cyclic (1.71±0.001 ppm) and anestrus heifers (1.55±0.09 ppm) than pubertal anestrus Sahiwal heifers. Significant difference (P<0.05) was observed in mean serum magnesium (mg/dl) and zinc levels in pre and post PRID-PG-GnRH and GnRH-PG-GnRH treated heifers. However, no significant difference was observed in pre and post BU exposed and control heifers. Umesh *et al.* (1995) reported similar serum magnesium level in normal cyclic (2.83±0.15 mg/dl) and anestrus cows (2.10±0.08 mg/dl). Dindorkar and Kohli (1979) found non-significant difference in serum magnesium levels in the regular cyclic cows.

From this study, it can be concluded that PRID-PG-GnRH and GnRH-PG-GnRH had significant effect on induction of estrus as reflected by increase in serum estradiol level after day 9 and followed by increase in progesterone level on day 10th post AI. Further, significant increase in serum calcium, magnesium, iron, and zinc levels during 30 days post treatment period might be associated with the resumption of estrus in pubertal anestrus Sahiwal heifers.

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