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Effect of GnRH treatment in augmentation of libido in relation to plasma androgens, thyroid hormones and biochemical profiles in poor libido breeding bulls

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Breeding bulls with poor libido (23%) create problem during semen collection and natural breeding (Kumar *et al.* 2008a) and this problem is also associated with poor semen quality and fertility (Ellis *et al*. 2005). Estradiol to testosterone ratio controls libido in breeding bulls (Singh *et al.* 2009, Kumar *et al.* 2008b), while leydig cells produce testosterone and convert to estradiol (Michael *et al.* 1987). Estrogen thus produced elicites masculine sexual behaviour in bulls (Roselli *et al*. 1985). Thyroxine (T_4) and tri-iodothyronine (T_3) regulate growth, metabolism of protein, carbohydrate lipid, and are also necessary for the expression of the changes in the neurosecretary system (Webster *et al.* 1991) thereby modulating reproduction phenomenon. Blood biochemical status indicate general health, normal metabolism and energy storage in the body (Amanullah 2009).

GnRH and LH are used for the treatment of poor libido bulls with variable success. However, information about the variations in plasma androgen, thyroid hormones and biochemical levels during the artificial enhancement of libido is lacking. Hence, the present study was conducted to assess the effects of exogenous GnRH treatment on libido in relation to variations in estradiol, testosterone, T_3, T_4 and biochemical profiles in breeding bulls affected with poor libido.

Crossbred (Holstein Friesian × Sahiwal) breeding bulls (8) between 3 and 18 years of age selected from an organized bull farm in Punjab were used for the experiment. Bulls were either progeny tested or under testing program, and semen was collected biweekly using artificial vagina. All the bulls had the history of excellent libido that declined gradually over a period of 4–6 months. Bulls were assessed for libido at the time of semen collection on the basis of reaction time. Breeding soundness evaluation was done to assess their structural and reproductive fitness. A looped measuring tape was kept around the greatest diameter of the scrotum and tape was pulled in such a way that it remained in close contact with the entire circumference. Testicular length and width was measured and testicular volume was calculated (Harriet *et al.* 2002).

All the bulls were given 3 injections of GnRH (buserelin acetate) intramucularly in decreasing dose, 20μg, 16μg and 12μg at an interval of 2 days. Blood samples were collected in vials containing heparin before treatment and 24 h after every GnRH injection. Two additional blood samples were collected after the last GnRH injection at an interval of 24 h. Harvested plasma was stored at ×20°C. Plasma estradiol, testosterone, T_3 and T_4 were estimated by ELISA kits. Glucose, cholesterol, triglyceride, total lipids, albumin and total protein was analyzed in plasma samples (before treatment) by automatic blood analyzer. Data were analyzed by ‘t’ test using analysis software SPSS Ver 16.0.

In the present study, the reaction time of breeding bulls remained between 10 and 15 min indicating poor libido. Breeding soundness evaluation did not reveal any defects in the musculoskeletal system, external or internal genitalia. The average scrotal circumference, left and right testicular volumes in poor libido bulls were 35.2 cm, 276.71 cm^3 and 282.32 cm^3, respectively, which were within the normal range of adult breeding bulls (Singh *et al.* 2010). Hence, breeding soundness evaluation based observations did not reveal any physical stressful conditions which may affect libido.

Out of 8 poor libido bulls, 5 bulls gained improvement in libido and 3 bulls failed to respond to GnRH treatment. The average estradiol to testosterone ratio in responding bulls decreased significantly after GnRH treatment (Table 1). However, in non- responding bulls the estradiol to testosterone ratio increased significantly. The higher estradiol to testosterone ratio in non-responding bulls indicated the differential conversion of testosterone to estradiol following GnRH therapy. Breeding bulls require threshold level of testosterone to exhibit their sexual activity (Blockey and Galloway 1978) and the GnRH administration increases the plasma testosterone through LH mediated stimulation of

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leydig cells. Hence, libido improvement in poor libido bulls might have occurred due to higher testosterone and no response in some of the bulls might have occurred due to higher estradiol following GnRH therapy. The GnRH induced increase in testosterone is well understood. However, GnRH induced decline in testosterone could be explained on the basis of focal enzymatic defects in steroid biosynthetic pathways. The decrease in androgen production following GnRH administration is due to the inhibitory effects of estradiol on 17 α hydroxylase and 17–20 desmolase activity (Bambino *et al.* 1980, Nozu *et al.* 1981), where they observed that intracellular estradiol produced after gonadotropin administration inhibits these enzymes and testosterone production is minimized (Cigorraga *et al.* 1980). The higher ratios of estradiol to testosterone in present study may be due to the enzymatic defects in androgen biosynthetic pathways, thereby modulating the variations in levels of plasma steroids in response to GnRH administration in bulls affected with poor libido.

The average T_3 to T_4 ratio in treatment responding bulls before treatment was 176.13±36.7, which increased nonsignificantly (P>0.05) (Table 2). However, in non-responding bulls, the average T_3 to T_4 ratio decreased significantly after treatment (P<0.05). In the present study the bulls which had lower T_3 to T_4 ratios failed to respond to GnRH treatment. It has been observed that hypothyroidism reduces the concentration of serum sex hormone binding globulin (SHBG) (Olivo *et al.* 1970), which might alter the plasma testosterone concentration (Ford *et al.* 1992) thereby affecting libido. Hypothyroidism is also associated with low basal metabolic rate (Hardman *et al.* 2001) and control of neurosecretary pattern in hypothalamus (Webster *et al.* 1991). Hence, lower T_3 to T_4 ratio induced lesser basal metabolic rates and changes in the neurosecretary pattern at hypothalamus might have reduced the therapeutic response of GnRH in poor libido bulls.

The overall average glucose, cholesterol, triglyceride, total lipids, albumin and total protein in treatment responding were 66±10.32 (mo/dl), 75.25±16.13 (mo/dl), 28.62±5.63 (mo/dl), 207.62±55.45 (mo/dl), 3.09±0.12 (g/dl) and 8.35±1.53 (g/dl), respectively. The respective values in non-responding bulls were 55±6.96 (mo/dl), 52.21±11.46 (mo/dl), 21.51±8.96 (mo/dl), 153.45±46.12 (mo/dl), 2.56±.75 (g/dl) and 6.71±2.31 (g/dl), respectively. The plasma glucose,

Table 1. Estradiol to testosterone ratios after intramuscular GnRH administration in poor libido bulls

Bulls	Before Rx (day 0)	first inj (day 1)	second inj (day 3)	third inj (day 5)	day 6	day 7	Libido status
1	1.77	1.2	2.34	6.82	1.96	1.17	Improved
2	11.11	0.48	0.72	13.33	3	0.44	
3	8.89	13.33	0.56	0.35	0.7	6.76	
4	12.89	7.52	1.22	4.52	2.12	2.31	
5	10.11	4.98	3.21	9.58	0.33	3.71	
Mean ±SE	8.95±1.91	5.50±2.34	1.61±0.50	6.92±2.20	1.62±0.49	2.87±1.11*	
6	6.57	55.35	3.0	2.8	4.17	18.57	No improvement
7	8.96	32.11	11.1	3.22	9.87	12.97	
8	9.92	21.23	7.82	6.97	12.3	14.56	
Mean ±SE	8.48±0.99	36.23±10.06	7.30±2.35	4.33±1.32	8.78±2.40	15.36±1.66*	*Indicates significant

(P<0.05) value prior to treatment**.**

Table 2. T_3 to T_4 ratios after intramuscular GnRH administration in poor libido bulls

Bulls	Before Rx (day 0)	first inj (day 1)	second inj (day 3)	third inj (day 5)	day 6	day 7	Libido status
1	316.66	566.66	552.63	689.81	633.33	589.74	Improved
2	102.17	185.71	100.00	180.00	278.50	554.05	
3	152.17	140.00	66.66	255.31	228.57	144.44	
4	145.56	389.45	88.45	204.67	245.56	349.67	
5	164.11	290.86	102.45	234.78	345.11	298.58	
Mean ±SE	176.13±36.7	314.53±76.6	182.03±93.1	312.91±95.3	346.21±74.6	387.29±83.0	
6	93.10	43.33	55.50	65.78	69.56	59.09	No improvement
7	72.19	27.55	56.1	75.34	80.56	42.33	
8	88.98	33.45	48.78	56.78	70.67	63.45	
Mean ±SE	84.75±6.3	34.77±4.6	53.46±2.3	65.96±5.3	73.59±3.4	54.95±6.4*	

*Indicates significant (P<0.05) value prior to treatment.

cholesterol, triglyceride and total lipids were significantly ($P<0.05$) higher in treatment responding bulls as compared to non-responding bulls. The blood glucose depends upon a wide variety of factors and equilibrium between its entry and removal in the circulation (Zelnicek 1968). Triglycerides are the most important lipids with respect to energy storage (Welson 2006). However, cholesterol and phospholipids are the important lipoid membrane structures. The plasma proteins act as a cell component and maintain osmotic pressure, acid base balance and body defense (Tatsapong *et al.* 2010). In the present study, higher values of lipids in treatment responding bulls indicate sound health that might have facilitated the bulls to respond to the GnRH treatment. It may be concluded from this study that estradiol to testosterone ratio and T_3 to T_4 ratio play an important role in exhibition of libido and the therapeutic response of GnRH. Higher plasma glucose, cholesterol, triglyceride and total lipids indicate a good health and facilitate the response of GnRH treatment. Three injections of GnRH (buserelin acetate) @ 20μg, 16μg and 12μg at an interval of 2 days improves libido in those bulls which have lower estradiol to testosterone ratio, higher T_3 to T_4 ratio and higher blood glucose, cholesterol, triglyceride and total lipids.

SUMMARY

The study was conducted to observe response of GnRH treatment on libido, plasma androgen, thyroid hormones and biochemical profiles in bulls affected with poor libido. Eight crossbred bulls affected with poor libido were selected for this experiment. All the bulls were given 3 intramuscular injections of GnRH @ 20μg, 16μg and 12μg at an interval of 2 days. In GnRH treatment responding bulls, the average estradiol to testosterone ratio decreased significantly. However, in non-responding bulls, average estradiol to testosterone ratio increased significantly. While the average T_3 to T_4 ratio in treatment responding bulls increased nonsignificantly. In non-responding bulls, the respective values decreased significantly. The plasma glucose, cholesterol, triglyceride and total lipids were significantly higher in GnRH treatment responding bulls as compared to non-responding bulls. It was concluded that 3 injections of GnRH @ 20μg, 16μg and 12μg at an interval of 2 days improved libido in bulls having poor libido.

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