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# Gonadal Steroids and Thyroid Hormones in Non-responding GnRH Treated Poor Libido Breeding Bulls

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## ABSTRACT

The study was conducted to assess the factors responsible for the no response of GnRH treatment in poor libido breeding bulls. Eight crossbred (Holstein Friesian x Sahiwal) breeding bulls with the history of gradual decline in libido over a period of 4-6 months from an organized bull farm in Punjab were selected. To rule out the possibility of physical stressful stimuli which may affect libido, breeding soundness evaluation of bulls was carried out. All the bulls were given 3 injections of GnRH (Buserelin acetate) in decreasing dose i.e. 20µg, 16µg and 12µg at an interval of 2 days. Blood samples were collected before treatment and 24 hrs after every GnRH injection. Plasma Estradiol (E), Testosterone (T), T3 and T4 were analyzed using Microwell ELISA Kits. Breeding soundness evaluation based observations did not reveal any defects in physical conformation and external or internal genitalia indicating absence of physical stressful stimuli which may affect libido. The E to T ratio increased from 6.57 to 44.28 in non responding bulls and decreased from 3.47 to 1.70 in responding bulls indicating differential conversion of T to E following GnRH therapy. Non responding poor libido bulls showed decline in T3 to T4 ratio from 90.0 to 64.54. However, T3 to T4 ratio increased from 227.65 to 293.36 in treatment responding bulls. It may be concluded that E to T and T3 to T4 ratios play an important role in modulating the therapeutic response of GnRH. Three injections of GnRH (Receptal, Buserelin) @ 20µg, 16µg and 12µg at an interval of 2 days improved libido in those bulls which had lower E, higher T, T3 and T4 following the GnRH treatment.

**Key words:** Gonadal steroids, Thyroid hormones, GnRH, Poor libido bulls.

## INTRODUCTION

Poor libido is a commonly found problem (23%) in breeding bulls which poses difficulty at the time of semen collection and natural breeding (Kumar *et al.*, 2007). This trait is also associated with poor semen quality and fertility (Ellis *et al.*, 2005) ultimately affecting financial profitability of the dairy farmers. Hence, there is need to develop a suitable therapeutic strategy for the treatment of poor libido in breeding bulls.

Preliminary studies indicate that estradiol (E) to testosterone (T) ratio controls libido in breeding bulls (Davidson, 1977). Leydig cells produce T which gets converted to E by aromatization in the sertoli cells, adipose tissues and hypothalamic pre-optic area (Michael *et al.*, 1987). E thus produced acts at hypothalamus to elicit masculine sexual behaviour in bulls (Roselli *et al.*, 1985). Further, thyroid hormones are closely associated with libido. In human, hypothyroidism is associated with poor libido or impotence (Wortsman *et al.*, 1987). Thyroxine (T4) and tri-iodothyronine (T3) not only regulate growth, protein, carbohydrate and lipid metabolism but also necessary for the expression of the changes in the neurosecretary system (Webster *et al.*, 1991) thereby modulating reproduction.

Various hormonal therapies (GnRH, LH) adopted for the treatment of poor libido bulls have met with variable success. However, there is little knowledge regarding the reasons of failure of the GnRH treatment. Hence, the present work was planned to assess the factors responsible for the non response of GnRH treatment on the basis of gonadal steroids and thyroid hormones.

## MATERIALS AND METHODS

Eight crossbred (Holstein Friesian x Sahiwal) breeding bulls between 3-18 yrs of age from an organized bull farm in Punjab were selected. Bulls were either progeny tested or under progeny testing program and semen was collected biweekly using artificial vagina. Bulls were maintained under loose housing system (covered area: 12 x 10 ft; uncovered area: 25 x 10 ft) and standard feeding schedule along with adlib

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green fodder. 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**

All the bulls were examined for structural soundness i.e. functional feet, associated joints, hooves and overall body conformation, external reproductive organs i.e. scrotum, testes, epididymis, prepuce and penis. Special examination included examination of internal reproductive organs such as pelvic urethra, prostrate gland, seminal vesicles, ampulla and measurement of scrotal circumference and testicular volume. Scrotal circumference was measured by holding the testicles firmly in to the lower part of the scrotum so as to minimize the scrotal wrinkles. A looped measuring tape was kept around the greatest diameter of the scrotum and tape is 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 by the formula for ovoid objects: Length x $Width^2$ x 0.522 (Harriet *et al.*, 2002).

**Hormonal treatment**

All the bulls were given 3 injections of GnRH (Buserelin acetate, Receptal, Intervet, Holland) in decreasing dose, 20µg, 16µg and 12µg at an interval of 2 days. Blood samples were collected in heparinized vials before treatment and 24 hrs after every GnRH injection. Two additional samples were taken at an interval of 24 hrs after the last treatment. Plasma was harvested and stored at -20$^0$C. Plasma E, T, T3 & T4 were estimated using Microwell ELISA Kits (Tanya Biotech, Mohali, Punjab).

## RESULTS AND DISCUSSION

In the present study, the reaction time of breeding bulls varied between 10-15 minutes 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, 275.21 $cm^3$ and 280.92 $cm^3$, respectively, falling within the normal range of adult breeding bulls. Hence, breeding soundness evaluation based observations did not reveal any physical stressful stimuli which may affect libido.

Out of 8 poor libido bulls, 5 bulls showed improvement in libido and 3 bulls failed to respond to GnRH treatment. Hormonal profile of E, T, T3 and T4 were quite different in GnRH non-responding and responding bulls. In non-responding bulls, E increased from 23 ± 12.1 to 31 ± 14.3 pg/ml and T decreased from 3.5 ± 2.20 to 0.56 ± 1.21 ng/ml following 1$^{st}$ GnRH injection and remained at similar level even after the treatment. Contrary to this, E decreased in responding bulls from 70 ± 12.1 to 53.8 ± 16.1 pg/ml and T increased from 20.14 ± 10.3 to 31.6 ± 12.2 ng/ml following the treatment. Since the ratios of E to T are more important than the individual values of the hormones in regulating libido (Singh, 2008) the ratio was calculated. The E to T ratio increased from 6.57 to 44.28 in treatment non-responding and decreased from 3.47 to 1.70 in responding bulls indicating differential conversion of T to E following GnRH therapy. Breeding bulls require threshold level of T to show their sexual activity (Blockey and Galloway, 1978) and the GnRH administration increases the plasma T through LH mediated stimulation of leydig cells. Hence, libido improvement in poor libido bulls might have occurred due to higher T and no response in some of the bulls might have occurred due to higher E following GnRH therapy. The GnRH induced increase in T is well understood. However, GnRH induced decline in T could be explained on the basis of focal enzymatic defects in steroid biosynthetic pathways. Decrease in androgen production following GnRH administration in rats suggested its inhibitory effects due to defects in 17 alpha hydroxylase and 17-20 desmolase activity (Bambino *et al.*, 1980; Nozu *et al.*, 1981). The defects in the enzymes are due to the inhibitory effects of intracellular E produced from androgen during the initial elevation of T secretion following gonadotropin administration (Cigorraga *et al.*, 1980). So, the decrease in T and increase in E may be due to the enzymatic defects in androgen biosynthetic pathways, thereby modulating the response of GnRH in poor libido bulls.

The T3 and T4 differed widely following GnRH administration in poor libido bulls. The average T3 and T4 in non-responding bulls varied from 135 ± 97 to 142 ± 68.2 ng/dl and 1.5 ± 1.1 to 2.2 ± 1.9 µg/dl during the treatment. However, the

average T3 and T4 in treatment responding poor libido bulls increased from 708 ± 72.1 to 923 ± 62.7 ng/dl and 3.11 ± 0.06 to 4.63 ± 1.13 µg/dl after 1st GnRH injection, which decreased subsequently to 619 ± 42.5 ng/dl and 2.11 ± 0.88 µg/dl, respectively, at the end of therapy. The T3 and T4 values were lower in non responding bulls as compared to responding bulls. Further, the analysis of T3 to T4 ratio revealed differences on the basis of response of GnRH treatment. Non responding poor libido bulls showed decline in T3 to T4 ratio from 90.0 to 64.54. However, T3 to T4 ratio increased from 227.65 to 293.36 in treatment responding bulls indicating a modulating role of T3 and T4 in GnRH response. 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.*, 1999). Hence, lower T3 to T4 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.

It may be concluded that E to T and T3 to T4 ratios play an important role in exhibition of libido and the therapeutic response of GnRH. The Poor libido bulls which did not respond to the treatment had higher E to T and lower T3 to T4 ratio following the treatment. Contrary to this, treatment responding bulls had lower E to T and higher T3 and T4 ratios. Three injections of GnRH (Receptal, Buserelin) @ 20µg, 16µg and 12µg at an interval of 2 days improved the libido in those bulls which have lower E, higher T, T3 and T4 following the treatment.

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