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Effects of Intravenous hCG Administration on Plasma Steroids in Breeding Sahiwal Bulls

Ajeet Kumar[1]*, Anand Kumar Pandey[2], Mrigank Honparkhe[1], Ankit Kumar Ahuja[3], Amarjeet Bisla[1] and Prahlad Singh[1]

[1]Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab
[2]Department of Veterinary Gynaecology and Obstetrics, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar.
[3]Department of Veterinary Clinical Complex, DGCN College of Veterinary and Animal Sciences, CSK Himachal Pradesh Agriculture University, Palampur, Himachal Pradesh

ABSTRACT

The present study was conducted to assess the response of intravenous administration of hCG on plasma testosterone, estradiol, dehydroepiandrosterone (DHEA) and estriol levels in breeding Sahiwal bulls. Six breeding Sahiwal bulls were injected intravenously with 1500 I.U hCG followed by blood sampling at an interval of every 15 minutes for 4 hours. Plasma testosterone, estradiol, DHEA and estriol were estimated and the values were calculated by elaborating the standard curve. It was observed that testosterone level declined within 15 minutes of hCG administration followed by gradual increase until 3h. Fluctuation among the DHEA levels at different time intervals were somewhat near to the basal levels and similar to testosterone. However, non-significant differences were observed in plasma estradiol and estriol following hCG administration. It was concluded that endocrine status of Sahiwal bulls could be assessed between 1.25 to 3 hours of 1500 IU of hCG administration.

Key words: hCG, Libido, Sahiwal bull, Steroid hormone, DHEA.

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INTRODUCTION

Low libido and poor semen quality among breeding bulls are the major problems encountered in bovine artificial breeding industry. Breeding bulls affected with low libido and poor semen quality creates problem in meeting the targets of frozen semen production. It has been observed that almost one fourth population (23%) of breeding bulls are having the problems of low libido and occasionally poor semen quality (Kumar *et al.*, 2008a). Endocrine profile of breeding bulls plays an important role in the manifestation of libido and regulating spermatogenesis in bulls (Toocheck *et al.*, 2016). It has been observed that testosterone to estradiol ratio is a differential feature between good and poor libido bulls (Singh *et al.*, 2016). Increased estradiol levels can affect libido as testosterone and estradiol are negatively correlated (Javed *et al.*, 2000). In fact, testosterone to estradiol ratio is more important than their

*Corresponding author.
E-mail address: ajeetvet@yahoo.com (Ajeet Kumar)
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individual values in regulating libido (Singh *et al*., 2015). The underlying reason is that leydig cells produce testosterone, which gets converted to estradiol by aromatization in sertoli cells, adipose tissues and hypothalamic pre-optic area (Michael *et al*., 1987). Increased aromatization of testosterone to estradiol leads to decreased testosterone to estrogen ratio, which might be the one of the reasons of poor libido in breeding bulls. On the other hand, in the absence of testosterone or functional androgen receptors, males are infertile due to impaired spermatogenesis (De Gendt *et al*., 2004). Various studies have been conducted to elucidate the normal levels of steroid hormone and or thyroid hormones in breeding bulls (Kumar *et al.,* 2008a; Shatab *et al.,* 2016).

For the therapeutic management of poor libido bulls, parenteral administration of gonadotropins has been carried out with variable success (Kumar *et al.,* 2008b; Kumar *et al.,* 2011; Monaco *et al*., 2015). Testosterone production in Sahiwal bulls increases exponentially with age as compared to HF × Tharparkar bulls (Gulia *et al*., 2010). In Egyptian pubertal bulls, administration of GnRH analogue, on a weekly interval, significantly elevates the reproductive health (El-Khawaga *et al*., 2011). Due to the anecdotal release of testosterone, its assessments require frequent blood collections over a period of time (Post *et al*., 1987). Perhaps, the importance of hCG stimulation prior to steroid analysis enables us to estimate the secretory capacity of testis to secrete them. Additionally, the fluctuation in the concentrations of steroid hormones like estradiol, DHEA and estriol post-hCG Stimulation has not been studied in Sahiwal bulls. Therefore, present study was conducted to assess the response of intravenous administration of hCG on blood plasma testosterone, estradiol, DHEA and estriol levels in breeding Sahiwal bulls.

MATERIALS AND METHODS

Selection of animals

The experiment was conducted on five Sahiwal cattle bulls, aged around 4-8 years, being maintained under a fully covered shed with in-group-loose housing system at the Govind Godham Goushala, Ludhiana, Punjab, India (30.9256° N, 75.7777° E). The bulls were clinically healthy with average body weight 525 ± 50 kg and were used for the natural breeding. The breeding bulls were negative for tuberculosis (TB), Johne's disease (JD) and brucellosis. As and when required, the bulls were shifted to female shed for natural breeding and thereafter shifted back to male section. The feeding schedule included 2.0 kg of concentrate mixture daily along with ad-lib green fodder.

Experimental protocol

A schematic representation of the experimental protocol is presented in Figure 1. The bulls were sedated with Inj. Xylazine (0.02 mg/kg body weight). An indwelling jugular venous catheter (16G, 4 inches) was fixed with skin by applying a knot with silk thread (No #1) about 30 minutes prior to the start protocol. All the bulls were injected intravenously with 1500 IU hCG (Chorulon®, MSD Animal Health, India). Blood samples (5ml) were collected into heparinized syringes at an interval of every 15 minutes for 4 hours. Blood samples were immediately transferred into heparinized tubes and placed on ice before centrifugation (5000 rpm for 10 minutes at 4°C). One blood sample was collected before the onset of treatment, which served as control. The separated plasma samples were stored at –20°C until hormone estimation.

Hormone estimation

On the day of hormone estimation, the plasma samples were ice thawed and vortexed uniformly. Plasma testosterone, estradiol, DHEA and estriol were estimated by 96 wells ELISA kits (Genxbio Health Sciences Pvt. Ltd., India) as per the kit manufacturer's instruction manual. The hormone values in samples were calculated by elaborating the standard curve with curve fitting system.

Statistical analyses

Homogeneity of variance was tested with repeated-measures analysis using the Generalized Linear Model procedure by SAS, 1999 software. Tukey's post hoc test was used to perform multiple statistical comparisons. The data were presented as mean ± SE. The significant interaction was considered at $P<0.05$.

RESULTS AND DISCUSSION

Present study was conducted to evaluate the relative changes in blood plasma steroid hormone levels following single intravenous injection of hCG (Chorulon®, 1500 IU, MSD Animal Health, India). The data were analyzed and presented as Table 1.Testosterone concentration declined immediately after the intravenous administration of hCG followed by gradual rise until in the last sample 3 hrs later. The mean basal concentration of testosterone before injection and in the last sample was 5.58±1.22 and 8.42±0.50 ng/ml, respectively. Increase in testosterone after hCG administration was observed among all the bulls. Testosterone

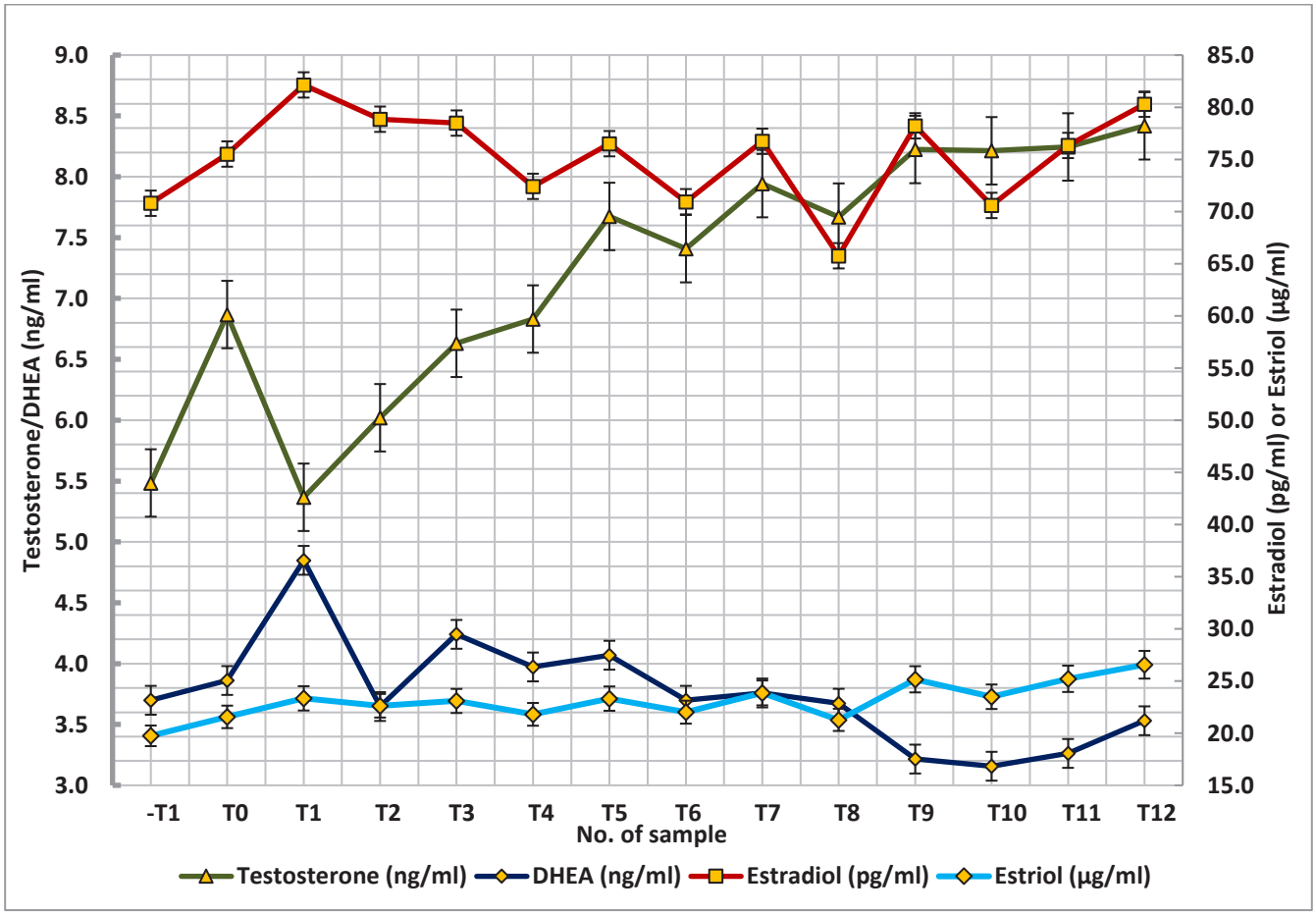

Fig. 1: Diagrammatic representation of experimental protocol

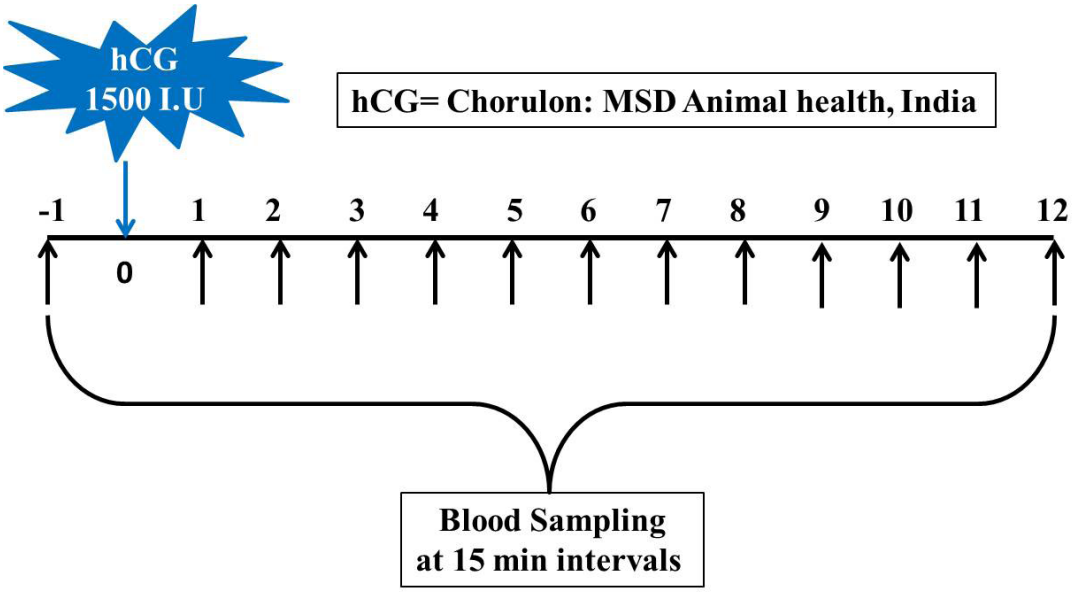

Fig. 2: Relative concentration of blood plasma hormones in Sahiwal bulls following intravenous hcG administration

Table 1: Blood plasma concentrations of testosterone, estradiol, DHEA and estriol following intravenous administration of hCG (1500 IU).

Hormones	Time points (Hours)														
	Before hCG	After hCG													
	11.00 (T-1)	11.15 (T0)	11.30 (T1)	11.45 (T2)	12.00 (T3)	12.15 (T4)	12.30 (T5)	12.45 (T6)	13.00 (T7)	13.15 (T8)	13.30 (T9)	13.45 (T10)	14.00 (T11)	14.15 (T12)	Coefficient of variation
Estradiol (pg/ml)	70.8 ± 20.7	75.5 ± 16.2	82.1 ±17.1	78.9 ± 17.83	78.5 ± 15.3	72.4 ± 17.7	76.5 ±15.9	70.9 ±18.1	76.7 ±17.9	65.8 ± 18.7	78.2 ± 17.7	70.6 ± 16.2	76.3 ± 18.7	80.3 ±18.3	52
Testosterone (ng/ml)	5.48 ± 1.23	6.87 ± 0.65	5.37 ±1.15	6.02 ± 0.76	6.63 ± 0.76	6.83 ± 0.86	7.67 ± 0.82	7.41 ± 0.92	7.94 ± 0.89	7.67 ± 0.70	8.22 ± 0.48	8.21 ± 0.44	8.24 ± 0.46	8.42 ± 0.50	24
DHEA (ng/ml)	3.70 ± 0.29	3.86 ± 0.29	4.85 ± 0.44	3.65 ± 0.34	4.24 ± 0.40	3.97 ± 0.50	4.07 ± 0.69	3.70 ± 0.42	3.76 ± 0.39	3.67 ± 0.41	3.19 ± 0.50	3.16 ± 0.47	3.26 ± 0.58	3.62 ± 0.40	26
Estriol (ng/ml)	26.2 ± 10.1	27.7 ± 6.47	21.4 ± 8.50	24.0 ± 8.23	25.1 ± 6.52	24.6 ± 10.1	24.8 ± 7.25	24.8 ± 9.73	24.4 ± 8.78	31.2 ± 7.37	25.7 ± 8.31	26.4 ± 10.9	30.3 ± 6.01	26.7 ± 8.13	72

levels attainted peak at 3 hrs post hCG administration. The difference between the values of T-1 and T0, and T0 and T12 was found significant statistically. The results show that testosterone level declines within 15 minutes of hCG administration followed by gradual increase until 3 hrs. The plasma estradiol rises to its peak concentration within 15 minutes of hCG administration and thereafter it remained at nadir up to 3 hours. Before hCG injection the concentration of estradiol was 70.8±20.7 pg/ml and in the last sample it rises to a level of 80.3 ±18.3 pg/ml. Non-significant differences were observed in plasma estradiol following hCG administration. The plasma dehydroepiandrosterone concentration rose immediately after the hCG injection and declined within 15 minutes to original level followed by maintenance at basal level up to 3 hrs of hCG injection. Before treatment, mean basal concentration of DHEA was 3.70±0.29 ng/ml and in the last sample it decreases to a level of 3.62 ±0.40 ng/ml. Similar to testosterone, DHEA level also rises immediately after hCG administration followed by a decline immediately and then gradually reached a peak. Fluctuation among the DHEA levels at different time intervals were somewhat near to the basal levels. The level of estriol remained unchanged throughout the treatment period in response to hCG administration.

In this experiment, we studied the effect of administering 1500 IU of hCG analogue on blood plasma levels of testosterone, estradiol, DHEA and Estriol. Our results show that hCG treatment significantly increased testosterone levels in Sahiwal bulls. A similar testosterone trend had previously been observed in GnRH-treated cattle bulls (Devkota *et al*., 2011) and GnRH-treated stallions (Roser & Hughes, 1992). The testosterone peak observed 2 hours after the gonadorelin injection in the dromedary camel is also similar to the testosterone peak observed after a single administration of GnRH in cattle bulls (Post *et al*., 1987). Further, the effects of different GnRH doses (from 20 µg to 600 µg) on bulls' testosterone levels revealed that time to attain peak were not dose-dependent. Consequently, a time window of between 2 to 3 hours post gonadotropin injection was proposed for measuring plasma testosterone levels in GnRH-treated bulls. Our study suggests that, following hCG injection, testosterone peaked within 30 minutes followed by sharp decline in Sahiwal bulls after hCG administration. The testosterone concentration remained plateau between 1.25 to 3h. Hence, this time could be used to assess blood testosterone levels post injection for clinical purpose. Threshold levels of testosterone is required to exhibit the sexual activity of breeding bulls (Blockey and Galloway, 1978) and administration of GnRH leads to rise of testosterone via LH mediated stimulation of leydig cells. Ratio of estradiol to testosterone is the determining factor that controls libido in breeding bulls (Kumar *et al*., 2008b), while leydig cells produce testosterone and convert to estradiol (Micheal *et al*., 1987). This estradiol production elicits the masculine sexual character and behavior in bulls. Decrease in androgen production after GnRH administration is due to inhibitory effect of estradiol on 17alpha hydroxalase and 17-20 desmolase activity (Bambino *et al*., 1980, Nozu *et al*., 1981). Due to intracellular production of estradiol following gonadotropin administration, these enzymes inhibited and testosterone production is minimized (Cigorraga *et al*., 1978). The effect of DHEA on sperm function has not been reported yet. DHEA acts as a substrate for testosterone synthesis *in vitro* in rabbits and are considered as a potential androgen (Prunty, 1966).

CONCLUSIONS

On the basis of this study, it was concluded that endocrine status of Sahiwal bulls could be assessed between 1.25 to 3 hours of 1500 IU of hCG administration.

CONFLICT OF INTEERST

None.

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