MARDI Res. J. 21(2) 1993: 145–150

Testosterone response to synthetic gonadotropin releasing hormone injection in Sahiwal-Friesian crossbred bulls

(Gerak balas testosteron terhadap suntikan hormon pengeluar gonadotropin tiruan pada lembu jantan kacukan Sahiwal-Friesian)

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Key words: GnRH, bulls, testosterone

Abstrak

Kajian ini dijalankan untuk menentukan kesan tujuh aras dos suntikan tunggal hormon pengeluar gonadotropin (GnRH) tiruan secara intraotot terhadap gerak balas testosteron di dalam plasma darah bagi lima ekor lembu jantan dewasa kacukan Sahiwal-Friesian yang berumur antara 23 dan 25 bulan. Dos GnRH yang digunakan ialah 0, 0.005, 0.01, 0.02, 0.04, 0.08 dan 0.16 µg/kg berat badan. Sampel darah diambil pada 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0 dan 7.0 jam selepas suntikan GnRH dari pukul 0900 hingga pukul 1600. Kajian ini mendapati bahawa dos GnRH yang rendah iaitu 0.005 µg/kg berat badan mencukupi untuk mengaruh testosteron mencapai puncak gerak balas maksimum. Meninggikan dos GnRH sehingga 32 kali ganda tidak mempengaruhi secara nyata (p >0.05) ketinggian puncak gerak balas testosteron dan keluasan geraf di bawah keluk gerak balas. Tempoh optimum untuk mengukur ketinggian puncak gerak balas testosteron adalah antara 2 jam hingga 3 jam selepas suntikan GnRH. Korelasi yang tinggi (r = 0.83, p <0.01) antara puncak gerak balas testosteron dan luas geraf di bawah keluk gerak balas dalam kajian ini menunjukkan bahawa pengambilan sampel darah tunggal yang dibuat pada masa yang sesuai ialah pilihan lain yang lebih baik daripada menentukan luas di bawah keluk gerak balas yang memerlukan satu siri pensampelan darah.

Abstract

A study was conducted to determine the effects of seven dose levels of a single intramuscular injection of a synthetic gonadotropin releasing hormone (GnRH) on plasma testosterone response in five Sahiwal-Friesian crossbred bulls aged between 23 and 25 months. The GnRH doses were 0, 0.005, 0.01, 0.02, 0.04, 0.08 and 0.16 µg/kg body weight. Blood samples were collected at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0 and 7.0 h post-injection of GnRH from 0900 to 1600 h. A small GnRH dosage of only 0.005 µg/kg body weight was observed to be adequate in inducing a maximum peak response in testosterone level. Increasing the dose of GnRH by a 32-fold range did not influence significantly (p >0.05) the height of testosterone response peak or testosterone area under the response curves. The optimal time to measure the height of the testosterone response peak was found to be between 2 h and 3 h post-injection of GnRH. The

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high correlation ($r = 0.83$, $p < 0.01$) between the testosterone response peak and area under the response curve showed that a single blood sample collected at an appropriate time might be a suitable alternative to the measurement of area under the response curve which needs a series of blood samplings.

Introduction

Testosterone, produced mainly by Leydig cells, is known to be essential for reproduction in males. Testosterone influences libido and is necessary for maintenance of serving capacity in bulls (Blockey and Galloway 1978). Testosterone is secreted in an episodic manner and there are about 4–10 peaks each day in plasma levels of testosterone in bulls (Katongole et al. 1971). Because of this episodic nature of testosterone secretion, attempts to relate plasma testosterone concentration with the reproductive performance of bulls were unsuccessful (Foote et al. 1976; Chenoweth et al. 1979). For a more accurate assessment of plasma testosterone concentration, a series of blood samples should be collected periodically over a period of time. However, such a method is not only time consuming but also unreliable because stress caused to the bulls during restraining and collection of blood by jugular venipuncture could restrain the secretion of testosterone during blood collection (Nancy et al. 1977). However, injection of exogenous gonadotropin releasing hormone (GnRH) was found to induce peak levels of plasma testosterone concentration in beef cattle (Post, Reich et al. 1987). In a recent study, it has been found that there was a significant correlation between GnRH-induced blood testosterone concentrations and fertility (percentage of non-return) in bulls used for artificial insemination (Andersson 1992).

This study was conducted to determine (i) the efficacy of a single intramuscular injection of a synthetic GnRH to induce peak levels of plasma testosterone and (ii) the effect of various doses of GnRH and bleeding times on the levels of plasma testosterone concentration in Sahiwal-Friesian crossbred dairy bulls.

Materials and methods

Five Sahiwal-Friesian crossbred bulls aged between 23 and 25 months were used in this study. They were housed in a conventional shed and kept under uniform feeding and management throughout the period of study. All the five animals were subjected to a series of blood samplings for 7 h, i.e. from 0900 h to 1600 h, without any GnRH injection (zero dose of GnRH), during the 'day 1' of the experiment. From the day 2, a single blood sample a day was collected at 0900 h from each of the five bulls for three consecutive days (day 2, 3, and 4). Two weeks later, in a Latin-square design, each of the five bulls received five different doses of a synthetic GnRH (Buserelin, Receptal, Hoechst AG Munchen) with an interval of 2 weeks. The doses were 0.01, 0.02, 0.04, 0.08 and 0.16 µg/kg body weight, injected intramuscularly as a single dose. Since the lowest dose was found to induce a high testosterone plasma level, the animals were subjected to a lower dose of 0.005 µg/kg body weight of GnRH at the end of the trial. Blood samples from the jugular veins were collected into 10 mL heparinised vacuum tubes at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0 and 7.0 h post-injection of GnRH from 0900 h to 1600 h. The blood samples were centrifuged and the separated plasma was kept frozen until analysed for testosterone by radioimmunoassay.

Plasma testosterone, in unextracted plasma samples, was analysed by a solid-phase ^{125}I radioimmunoassay technique using RIA kits purchased from Diagnostic Products Corporation, USA. In this assay, 50 µL of buffer, standard (range from 0.2 ng/mL to 16 ng/mL) or plasma samples were added in duplicate into 12 mm x 75 mm polypropylene tubes coated with antibody to testosterone. One milliliter of

^{125}I testosterone was then added to each tube and total count tubes, and immediately vortexed for a few seconds. The tubes were later kept in a water bath at 37 °C for 3 h. Following that, all the tubes, except for total count tubes, were decanted and kept inverted until dry. The activity of ^{125}I was then determined for 1 min using gamma counter (ICN, Isoflex gamma spectrometer, USA) and the concentration of testosterone was automatically calculated using a programme that comes with the counter. The sensitivity of the assay was 0.19 to 16.50 ng/mL and the intra and inter-assay coefficient of variation of the assay were less than 5%.

Testosterone levels were measured as maximum heights of testosterone peaks or as areas under the curves to estimate the testosterone response. The areas under the response curves were calculated according to Post, Reich et al. (1987). For this purpose, a series of trapeziums were formed by drawing vertical lines from each point on the curve to the x-axis of the plotted curve. The area [area = 0.5 (side a ± side b) x distance between sides] of each trapezium was calculated. The sum of the areas of the series of trapeziums was taken as the area under the response curve. The data of the 0 min post-injection were excluded in calculating testosterone areas under the response curves. For statistical analyses, analysis of variance and regression analysis were used to calculate statistical differences.

Results

The mean plasma testosterone concentration of the five bulls with zero dose of GnRH at zero hour blood sampling was 9.44±0.92 ng/mL on day 1 and the concentrations were 4.34±1.00, 6.36±1.68 and 5.07±1.10 ng/mL on day 2, 3 and 4, respectively. The mean difference was significant (p <0.05) *(Table 1)*.

Table 1. Variations in the plasma testosterone concentration of Sahiwal-Friesian bulls without GnRH injection at 0900 h blood sampling

Days of sampling	No. of samples	Plasma testosterone concentration (ng/mL)
Day 1	5	9.44±0.92a
Day 2	5	4.34±1.00b
Day 3	5	6.36±1.68c
Day 4	5	5.07±1.10bc

Mean values (± SE) with different letters are significantly different (p <0.05)

The effects of GnRH doses on the testosterone response are shown in *Figure 1*. It was observed that in all the bulls from the zero-dose GnRH treatment, there was a sharp declining trend in the plasma testosterone concentration from 0 h to 3 h of blood samplings. Thereafter, there were random episodic testosterone peaks which were lower than the original peaks (at 0 h) with no consistent pattern among the bulls.

Increasing the dose of GnRH by a 32-fold range, i.e. from 0.005 µg/kg to 0.16 µg/kg body weight, did not have much influence on the testosterone responses. Statistical analysis showed that the height of testosterone response peak and testosterone area under the response curves did not differ significantly (p >0.05) among the six doses of GnRH *(Table 2)*. Though the mean testosterone peak of the zero dose was found to be lower than the testosterone response peaks of the other six GnRH doses, the difference was not statistically significant (p >0.05). However, the testosterone area under the response curve of the zero dose was significantly lower (p <0.01) than the testosterone area under the response curve of the other six GnRH doses.

The mean testosterone peak response over all the 30 doses of GnRH treatment showed that the peak response was reached at 2 h post-injection of GnRH. However, the mean plasma testosterone concentration remained almost uniform at 1.5, 2, 2.5 and 3 h *(Figure 1)*. After 3 h, there was a general trend of declining levels of plasma testosterone concentration returning to baseline levels.

There was a significant and positive correlation (r = 0.83, p <0.01) between

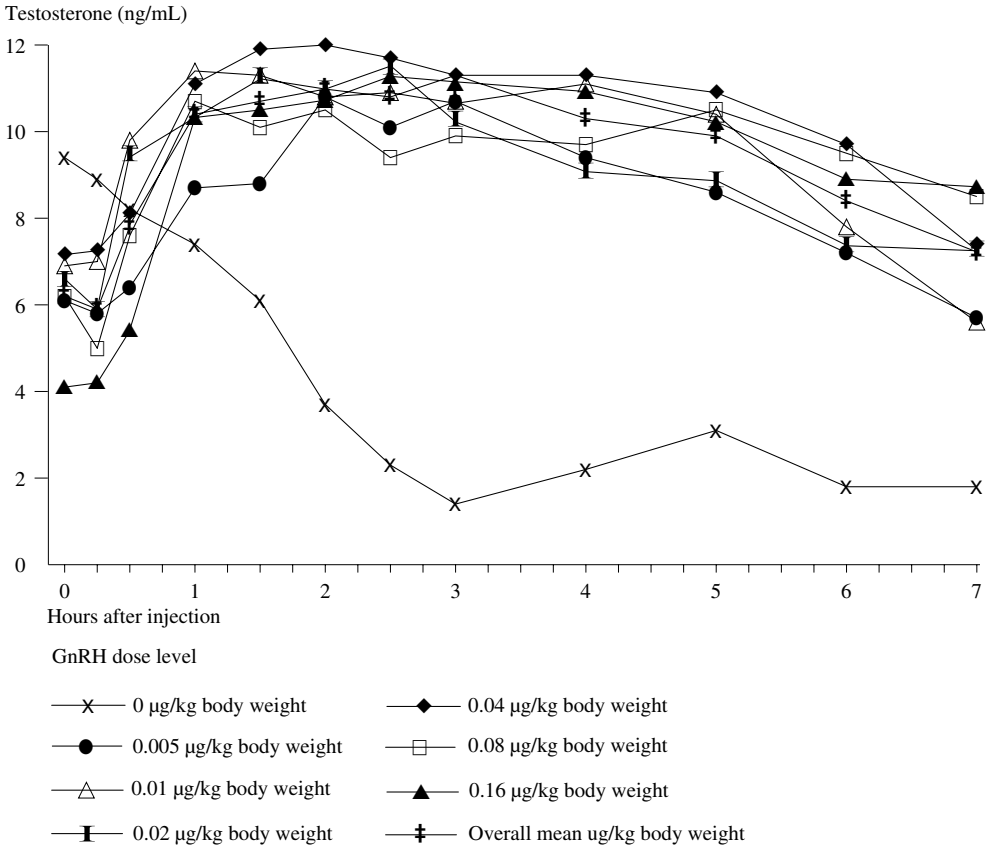

Figure 1. Mean testosterone responses to seven doses of GnRH injection in five bulls and the overall mean testosterone response

Table 2. Effects of seven GnRH doses on testosterone response peak and area under the response curve in Sahiwal-Friesian bulls

GnRH dose (µg/kg body weight)	No. of samples	Peak (ng/mL)	Area under the response curve
0	5	9.44±0.92a	23.07±3.33a
0.005	5	10.83±1.14a	59.34±6.33b
0.01	5	11.32±1.26a	66.23±4.22b
0.02	5	11.52±0.86a	62.02±4.32b
0.04	5	11.98±1.08a	71.29±5.62b
0.08	5	10.54±1.08a	65.67±2.19b
0.16	5	11.25±1.36a	66.71±5.06b

Mean values (± SE) in the same column with different letters are significantly different (p <0.01)

testosterone response peaks and the area below the response curves *(Table 3)*. However, the testosterone response peaks did not correlate significantly with the plasma testosterone concentration in the seven h GnRH post-injection blood samples.

There was no significant variation (p >0.05) among animals regarding the time at which the plasma testosterone concentration reached the peak, testosterone concentration at response peak and the area below the response curve *(Table 4)*.

Discussion

The present study compared the variation in plasma testosterone response to seven doses of GnRH treatments. The significant difference (p <0.05) among the mean plasma testosterone concentration on day 1, 2, 3 and 4 without any GnRH injection showed that there was no consistent daily pattern of testosterone secretion in the bulls. The sharp declining trend in the plasma testosterone concentration from zero to 3 h of bleeding for the zero dose could be due to the reaction of the bulls to the stressful stimuli such as restraining and collection of blood by jugular venipuncture. Similar findings were also reported by Nancy et al. (1977) on Angus bulls. The present results demonstrated that a single or sequential collection of blood from bulls cannot be used to evaluate normal temporal variations in plasma testosterone concentration.

It is interesting to note that although there was a 32-fold increase in GnRH dosage, there was no significant increase (p >0.05) in the testosterone response peak or testosterone area under the response curves. This showed that a small GnRH dosage of only 0.005 µg/kg body weight is enough to induce a maximum peak response in testosterone in bulls. This study further showed that the optimal time to measure the height of the testosterone response peak was between 2 h and 3 h post-injection for the smallest dosage of GnRH (0.005 µg/kg). For larger doses of GnRH, blood samplings could be obtained between 1.5 h and 3 h post-injection.

Table 3. Correlations between testosterone response and area below the response curve (ABRC) and plasma testosterone concentration at 7 h post-injection bleeding (7PTC)

	Correlation coefficient *(r)*	Significance	d.f.
Testosterone peak vs. ABRC	0.83	p <0.01	28
Testosterone peak vs. 7PTC	0.26	ns	28

ns = not significant (p >0.05)
d.f. = degrees of freedom

Table 4. Mean testosterone response over six doses of GnRH in Sahiwal-Friesian bulls

	No. of samples	Peak time (h)	Peak (ng/mL)	Area under the response curve
Bull 1	6	2.5	10.34±0.95	60.37±3.12
Bull 2	6	2.0	10.38±1.33	63.22±4.44
Bull 3	6	2.5	12.08±0.73	69.73±5.04
Bull 4	6	2.0	12.07±0.97	60.72±2.31
Bull 5	6	2.5	11.97±1.09	71.99±5.16
Animal diff.		ns	ns	ns

ns = not significant (p >0.05)

Since the height of the testosterone response peak was highly correlated ($r = 0.83$, $p < 0.01$) with the area under the response curve, a single blood sample collected at an appropriate time might be a suitable alternative to the measurement of the area under the response curve which needs a series of blood samplings. The non-significant coefficient of correlation ($p > 0.05$) observed between the testosterone response peak and the plasma testosterone concentration in the 7 h post-injection blood samples showed that testosterone response peak did not have any significant influence on the declining trend in the levels of plasma testosterone concentration returning to baseline levels after the peak height.

Analysis of variance showed that there was no significant variation ($p > 0.05$) among bulls in this study for the mean testosterone response peak and the area below the response curve. This could be due to the uniformity in the age (24.2±0.44 months), body weight (300.40±4.77 kg) and scrotal circumference (31.86±0.46 cm) of the bulls in the experiment as similarly reported by Mackinnon et al. (1991).

The information obtained from this study showed that the testosterone status of bulls could be easily assessed by a single blood sample collected between 2 h and 3 h after injecting intramuscularly a small dose (0.005 µg/kg body weight) of synthetic GnRH. Testosterone was found to be critical in libido and sperm maturation (Mann and Lutwak-Mann 1981). Post, Christensen et al. (1987) found that there was an agreement between the rankings for testosterone maximum level and reproductive performance, especially fertility, in three Zebu crossbred bulls and in four Hereford-Shorthorn bulls. Therefore, the results obtained from this study could help in developing a simple test for detecting bulls at an early age for low reproductive potential due to testosterone deficiency.

Accepted for publication on 26 October 1993

Acknowledgement

The authors are grateful to Mr Ahmad Shokri Othman for statistical analysis.

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