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RELATIONSHIP AMONG TESTOSTERONE RESPONSE TO GnRH ADMINISTRATION, TESTES SIZE AND SPERM PARAMETERS IN HOLSTEIN-FRIESIAN BULLS

G. Gábor,[1] M. Mézes,[2] J. Tőzsér,[2] S. Bozó,[1] E. Szűcs[2] and I. Bárány[1]

[1] Research Institute for Animal Breeding and Nutrition, Herceghalom, Hungary
[2] Gödöllő University of Agricultural Sciences, Gödöllő, Hungary

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ABSTRACT

The investigation of the fertility capacity of A.I. bulls is one of the most important factors in their genetic improvement. The aim of this study was to examine the relationship between the results of the GnRH test and the measured and calculated size of the testicles and sperm parameters (sperm density and velocity, percentage of live and motile spermatozoa). Data was collected during three experiments from a total of 81 Holstein-Friesian breeding bulls at the National Artificial Insemination Centre of Hungary. In Experiment 1. a very close correlation ($r=0.64$, $p<0.001$; $r=0.66$, $p<0.001$; $r=0.99$, $p<0.001$; $r=0.75$, $p<0.001$) was found between the size of testes (weight, volume, width, length) and the calculated volume. According to the data, we may calculate volume based on the width of testicles measured by sonography.

In Experiment 2. the sizes of the testicles were compared with the results of the GnRH response test. There was a close ($r=0.63$, $p<0.05$) correlation between the volume of testicles and the GnRH induced serum testosterone levels.

In Experiment 3. it was shown that the GnRH test gives objective results only when repeated and with bulls in service. No significant correlation was found between the serum testosterone levels (before and after GnRH stimulation) and the sperm parameters.

Key words: bulls, testes, GnRH-stimulation, testosterone, sperm parameters

INTRODUCTION

It is desirable that bulls selected to be herd sires not only should show good reproductive performance themselves, but should pass the traits to their male offspring. Certain criteria were found to be useful for predicting these traits. Thus, Coulter and Kozub (4) demonstrated that a selection of herd sires with a large scrotal circumference,

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low backfat thickness, and low levels of primary sperm defects improved the fertility of beef bulls used under extensive range conditions.

Braun et al. (2) revealed that the potential value of testosterone and LH secretion after the administration of GnRH, is an indicator of semen quality in adult bulls.

In adult rams scrotal circumference correlated well with most physical and ultrasonic measurements and with the motility and percentage of abnormal spermatozoa (3). There were, however, significant differences between the physical and ultrasonic measurements of testis circumference and diameter, especially when ultrasonic measurements were made through the intact scrotal wall.

The aim of this study was to combine the above mentioned methods and to examine the relationships:

between estimated size and volume of the testes,
between the volume of testes and the results of the GnRH stimulation,
the effects of repeated GnRH challenges, and
the relationships between the results of repeated GnRH test and common sperm parameters.

MATERIALS AND METHODS

Our investigation was carried out on a total of 81 Holstein-Friesian bulls, located at the National Artificial Insemination Centre in Gödöllő. The bulls were 5 to 6 y old and had been used for artificial insemination at the Centre. They were housed in stables at a moderate (spring) temperature and fed a standard diet of 3.5 kg concentrate and alfalfa hay ad libitum. All the bulls were out of service (waiting bulls) except five bulls during Experiment 3. At the beginning of the study the bulls were randomly assigned to 3 experimental groups. The unequal group sizes were the result of the actual selection program at the A.I. Centre.

Experiment 1

The objective of this experiment was to investigate the relationships among the estimated sizes and the predicted and measured volumes of testicles. Bulls in Group 1 (n= 23) were slaughtered, the testes were harvested immediately and the spermatic cord and epididymis were removed. Each testis was weighed on an analytical balance and the diameter and length were measured with a sliding caliper. Actual testicular volume was determined by water displacement. The predicted testicular volume (PTV) was determined by 3 methods: Method 1, according to Toelle (17) was PTV = (testes width/2)^2 x testes length/2)^2. The formula for Method 2 according to Lunstra et al. (8) was PTV = 0.0396125 x (testes length) x (scrotal circumference)^2, where the scrotal circumference (SC) was calculated by the formula $SC=4r+2\pi r$. Method 3 was based on our calculations, developed as a result of a series of measurements taken on bulls of the same genotype. Values were calculated based on our own unpublished data. The

resulting formula was PTV = K x (testes width), where K = 45.63, was a constant derived from the mean measured testes volume/mean measured testis diameter using a sliding caliper. The mean measurements were taken from the same 23 bulls with both testes in duplicate.

Experiment 2

The objective of this experiment was to study the relationship among estimated size, the measured volume, the PTV and the results of the GnRH test. Bulls in Group 2 (n = 14) were given a GnRH analogue ®Ovurelin ad us. vet., 100 µg, im, (Reanal, Budapest, Hungary). Blood samples (20 ml) were taken from the jugular vein into test tubes just before and 90 min after the administration of GnRH as described by Wekerle et al.(19). The samples were stored at room temperature, until centrifuged and the serum was then poured off and stored in vials at -20 ^{o}C until assayed for testosterone. The bulls were slaughtered one day after the GnRH test. Testes were harvested and processed as described for Group I. The PTV was estimated as described in Method 3. Serum testosterone was assayed using a ^{125}I-labelled steroid assay kit (Institute of Isotopes,. Budapest, Hungary) following diethyl ether extraction (10), The sensitivity of the assay was 0.5 nmol and the within and between coefficients of variation were 6.3% and 9.2%, respectively.

Experiment 3

The objectives of this experiment were to investigate the effect of the repeated GnRH test on testosterone response and the relationship among the results of the repeated GnRH test, the sperm parameters and the size of testicles. In this study, the bulls (n = 44) were given 3 doses of GnRH (100 µg) im. In order to avoid possible down regulation by repeated doses of GnRH, the intervals between the first and second and the second and third dose were 4 and 6 wks, respectively. The most of these bulls (except five) had not been used for service for I yr. before the start of the study (waiting bulls). An ejaculate was collected with an artificial vagina prior to the start of the experiment to remove aged sperm. Subsequent ejaculates were collected with an artificial vagina 24 h later, after the first dose of GnRH, and subsequently once weekly for 10 wks. Sperm concentration and velocity were measured by an HTM 2000 motility analyser (IMV, L'Aigle, France). The PTV was estimated as described in Method 3, except that the bulls were not slaughtered and testes width was measured in vivo by a B-mode ultrasonograph (ALOKA Echo Camera, SSD-210 DX II) with a 5 MHz linear array (UST-5813-5, Aloka Ltd, Japan).

Data were analyzed by bivariate regression and correlation analysis (15) using a computer program software (Statgraphics, Statistical Graphics System, STSC Inc. and Statistical Graphics Co., U. S.).

RESULTS AND DISCUSSION

Experiment 1

The measured and calculated parameters of the testicles can be seen in Table I. The weight of the testicles shows a close correlation with their size (length and width) and also with their volume. The measured and calculated volume was in close or very close correlation, especially when the calculation was based on the width of the testicles (Table 2). The method presented here, of multiplying the testicular width by 45.63, is much simpler than the other methods. The calculated value was used for further calculation of correlation tests, because estimates with fewer parameters in order to avoid errors in measurements. Scrotal circumference is the most widely used method for the prediction of testis volume but this parameter depends a great deal on the age and breeding frequency of the bulls (4); thus, we were trying to develop a more objective method. However the low correlation between the calculated and measured volume in the present study might be caused by the different standard deviations of measurements taken in vivo compared to those taken post mortem. This is probably the reason for the marked difference between our results and those reported in previous studies (12).

Experiment 2

Among the measured and calculated parameters of the testicles, the weight and measured volume appear to affect the level of serum testosterone after GnRH stimulation. The correlation values in this studiy support (Table 3) our previous results (18) in that there was a close correlation between the increased testosterone content in the serum and the testosterone level after GnRH treatment (Table 1).This correlation may provide information about the responsiveness of Leydig-cells to LH. Perry et al. (13) suggested that the most important traits in fertility are the peripheral LH levels after GnRH stimulation and the testicular volume. In our opinion, the serum testosterone level depends on the number of Leydig cells (maximal testosterone producing capacity of testis). Fertility indices, in the form of multiple regression equations using pregnancy rate as a dependent variable, were derived from production and reproduction traits of beef bulls in Perry's study (13). Fertility indices showed significant multiple correlation among and within genotype ($P < 0.01$) at 11, 8, and 6 months prior to mating, $r=0.75$, $r=0.89$, $r=0.80$, respectively. It was found that the most important traits to include in the fertility indices were peripheral LH levels following GnRH stimulation, testicular volume, libido and body weight.

Experiment 3

Although large differences were found among actual serum testosterone concentrations after repeated GnRH stimulation (Table 4), the increase in response to the second and third GnRH dose was remarkably similar. Tannen and Convey (16) described that a series of GnRH injection may be more useful than a single dose in evaluating the capabilities of the pituitary to release LH. It was also found that chronic

Table 1. Size of testes measured by different methods (Experiment 1) serum testosterone and sperm parameters levels before and after a single (Experiment 2) and a repeated (Experiment 3) GnRH challenge

	Experiment 1 n = 23	Experiment 2 n = 14	Experiment 3 n = 44
Parameters	Mean ± SD	Mean ± SD	Mean ± SD
Average weight of 1 testis (g)	371.2 ± 68.8	-	-
Weight of 2 testes(g)	-	766.8 ± 115.7	-
Width of 1 testis (cm)	7.58 ± 0.71	7.5 ± 0.6	7.52 ± 0.5
Length of 1 testis (cm)	12.76 ± 1.13	12.6 ± 1.2	-
Measured volume of 1 testis (cm^3)	347.3 ± 67.8	-	-
Measured volume of 2 testes (cm^3)	-	715.7 ± 111.4	-
Calculated volume of 1 testis (cm^3) according to Toelle	294.3 ± 81.6	-	-
Calculated volume of 1 testis (cm^3) according to Lunstra	392.6 ± 108.7	-	-
Calculated volume of 1 testis based on width (cm^3)	345.8 ± 32.3	341.6 ± 26.2	344.4 ± 23.5
Base level of serum testosterone (nmol/l)	-	8.6 ± 5.5	8.7 ± 7.5
Serum testosterone after GnRH challenge (nmol/l)	-	21.6 ± 9.0	20.9 ± 9.4
Difference between base and induced level (nmol/l)	-	13.0 ± 7.6	12.1 ± 6.6
Total number of sperm cells in an ejaculate (10^9)	-	-	12.7 ± 3.8
Average speed of sperm cells in ejaculate (µm/sec)	-	-	93.2 ± 23.8
Live spermatozoa (%)	-	-	46.9 ± 13.4
Motile spermatozoa (%)	-	-	76.6 ± 12.1

Table 2. The results of regression analysis in Experiment 1 (Weight, measured and calculated volume of testes, n=23)

PARAMETERS		1	2	3	4	5	6	7
Average weight of 1 testis (g)	1	1						
Width of 1 testis (cm)	2	0.64 ***	1					
Length of 1 testis (cm)	3	0.54 ***	0.75 ***	1				
Measured volume of 1 testis (cm^3)	4	0.97 ***	0.66 ***	0.56 ***	1			
Calculated volume of 1 testis (cm^3) Toelle	5	0.67 ***	0.97 ***	0.85 ***	0.69 ***	1		
Calculated volume of 1 testis (cm^3) Lunstra	6	0.67 ***	0.97 ***	0.85 ***	0.69 ***	0.99 ***	1	
Calculated volume of 1 testis based on width (cm^3)	7	0.64 ***	0.99 ***	0.75 ***	0.66 ***	0.97 ***	0.97 ***	1

*** = $p<0.001$.

Table 3. The results of regression analysis in Experiment 2 (Size of testes, serum testosterone after GnRH administration, n=14)

PARAMETERS		1	2	3	4	5	6	7	8
Average weight of 2 testes (g)	1	1							
Width of 1 testis (cm)	2	0.5 NS	1						
Length of one testis (cm)	3	0.32 NS	0.68 ***	1					
Measured volume of 2 testes (cm^3)	4	0.98 ***	0.51 *	0.31 NS	1				
Calculated volume of 1 testis based on width (cm^3)	5	0.50 NS	0.99 ***	0.68 ***	0.51 NS	1			
Basic level of testosterone in blood sera (nmol/l)	6	0.45 NS	0.34 NS	0.03 NS	0.43 NS	0.34 NS	1		
GnRH induced level of testosterone in blood sera (nmol/l)	7	0.58 *	0.24 NS	0.11 NS	0.63 *	0.24 NS	0.54 *	1	
Difference between basic and increased level (nmol/l)	8	0.37 NS	0.04 NS	0.11 NS	0.43 NS	0.04 NS	-0.08 NS	0.79 ***	1

* = $p < 0.05$ *** = $p<0.001$.

Table 4: Serum testosterone concentrations in response to repeated GnRH stimulation of bulls (n=44)

	Testosterone (nmol/l) Mean ± SD			
Experiment	Before GnRH injection(A)	90 min. after GnRH injection(B)	Increase between A and B	Correlation between A and B
1st GnRH challenge (Day 0)	16.1±14.1	49.0±28.5	32.8±26.8	0.36 *
2nd GnRH challenge (week 4)	10.4±10.0	22.6±11.8	12.1±10.2	0.57***
3rd GnRH challenge (week 10)	6.9±6.7	19.2±10.0	12.3±6.9	0.73***
Mean of 2nd and 3rd GnRH challenge	8.7±7.5	20.9±9.4	12.1±6.6	0.72 ***

* = $p < 0.05$ *** = $p<0.001$.

administration of GnRH resulted in the depression of plasma testosterone; normal patterns returned within 60 days (7). Our results after the first GnRH test suggest that not only the frequency of GnRH treatment but the previous break in semen collection and/or service may play a role in testosterone response. This was supported by the results of our 2nd and 3rd tests where there were practically the same differences between the initial and stimulated levels of testosterone in the blood sera (Table 1) while in the first test we found higher values (Table 4). The comparison of the average results of the GnRH challenge (2nd and 3rd tests) and the semen parameters showed moderate or a lower than moderate correlation (r=0.02-0.16, non significant). These results suggest that the spermatogenesis is regulated by a more complex mechanism than testosterone alone and on the other hand, the collection of semen and the ejaculation frequency (besides accessory sexual glands) have a profound effect on ejaculation volume. This hypothesis is supported by Malak and Thibier (9) who found no significant correlation between the LH response to GnRH and semen production. The results of Asem (1) regarding the correlation between testicular and semen volume ($r=0.30$, $p<0.05$) were compared.

A close correlation was also found among the sperm quality parameters (e.g. live spermatozoa vs. motile spermatozoa $r=0.60$ $p<0.001$; sperm velocity vs. live spermatozoa $r=0.43$ $p<0.01$; sperm velocity vs. motile spermatozoa $r=0.38$ $p<0.01$; and the total number of sperm cells per ejaculate vs. motile spermatozoa $r=0.35$ $p<0.05$).

In conclusion, the precise measurements of testes may be determined by B-mode echograph for the characterisation of their real actual size and predicted volume based on width alone (see Method 3.).

The GnRH challenge only provides valuable results on the testosterone producing capacity of testis when repeated, and with bulls in service. In future experiments, we plan to use sonography to determine the ultrasonic image of testes and compare these results with the blood testosterone level after the GnRH test.

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