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ULTRASONOGRAPHY OF TESTICULAR MEASUREMENTS IN RELATION WITH SEMEN QUALITY OF FRIESIAN BULLS TREATED WITH L-TYROSINE

Abu El-Hamd M. A. and Sh. M. Shamiah
Animal Production Res. Inst., Agric. Res. Center, Dokki, Giza, Egypt.

ABSTRACT

The objective of the present study was to evaluate treated with oral single dose of L-tyrosine (50 g/bull), on semen quality in relation with ultrasonograms pixel intensity and conventional measurements of the testes of Friesian bulls. Twenty Friesian bulls averaging 8.0±0.24 month of age and 195±15.6 kg body weight were used in this study. The experimental bulls were divided into two similar groups (10 each). Bulls in the 1st group were fed a basal diet and considered as a control group (G1), while those in 2nd group were received an oral dose from L-tyrosine (50 g/bull) at 8.5 months of age. Throughout the experimental period, the percentages of progressive motility, livability and abnormalities of spermatozoa as well as sperm cell concentration were evaluated. At 15 and 20 months of age, testicular breadth, thickness of scrotal layers and seminal cord, the testicular mediastinum thickness and pixel intensity value were recorded using ultrasound scanner. Results showed that testicular breadth, thickness of scrotal layers and testicular cord of the bulls were not affected significantly by tyrosine treatment. However, testicular breadth of the bulls at 20 month was higher than at 15 month of age. Bulls treated with L-tyrosine with the different age did not affect the thickness of testicular mediastinum. However, values of testicular pixel intensity was higher (P<0.01) in treated than in control bulls and at 20 than 15 month of age. Percentages of progressive motility, livability of spermatozoa, and sperm cell concentration of the bulls treated with L-tyrosine were significantly (P<0.01) increased and decreased (P<0.01) sperm abnormalities (10.97 *vs.* 30.35%) as compared to the control. At 20 month of age, sperm motility and livability as well as sperm cell concentration increased (P<0.01) by about 33.25, 22.52 and ٤٢,٨% and sperm abnormalities decreased (P<0.01) by 49.4% as compared to those at 15 month of age. The correlation coefficients between testicular pixel intensity and each of semen characteristics studied were highly significant (P<0.001), being positively the strongest with sperm livability (r= 0.7978), sperm cell concentration (r= 0.7243) and negatively with sperm abnormalities (r= -0.7978),. However, positively lower correlation was recorded between pixel intensity and sperm motility (r= 0.5493).

Keywords: Friesian bulls, L- tyrosine, testis, semen quality, testicular pixel intensity.

INTRODUCTION

The use of some organic substances, possessed to improve the growth performance and reproductive efficiency of animals, was suggested to enhance the release of sex hormones. A semi essential amino acid (L-Tyrosine) is one of these substances, which is involved in the formation of catecholamine from adrenal gland and thyroxin from thyroid gland (Hammel and Russe, 1987). In this respect, Abo-Elroos (1992) reported that organic substance L-Tyrosine possessed to improve the reproductive efficiency of the animal, through enhancing the release of sex hormones. Gabr (2009) found

that the double oral dose from L-Tyrosine containing 100 mg/kg live body weight (LBW) at 15 day-interval induced early puberty of rabbit bucks.

Previously, the assessment of male reproductive status has been done through histology (Curtis and Amann, 1981) or through monitoring testicular growth with scrotal circumference (Coulter and Foot, 1979). However, information about *in situ* physiological status of the testis is not available. Physiological status of the testis can be assessed in more detail by ultrasonography, which is very useful technique in imaging reproductive organs of farm animals (Griffin and Ginther, 1992) and this technique is well described for reproductive status of female cattle (Pierson *et al.*, 1988).

The ultrasonographic image is composed of an array of pixel (picture elements) and the pixel intensity varies depending upon the characteristics of the tissue (relative density) examined (Graffin and Ginther, 1992). The testicular transcutaneous ultrasound images may provide more useful information than measuring scrotal circumference in bull (Pechman and Eilts, 1987). Visual assessment of testicular ultrasonographic image in the absence of gross pathological conditions has very limited diagnostic value since there are no significant correlation between visual analysis and semen quality in bulls (Kastelic *et al.*, 2001). Use of computer assisted analysis has added new power to reproductive ultrasonography. This analysis gives us pixel, which increase analysis value of ultrasnographic images and can provide substantial information regarding the function and structure of tissue (Pierson and Adms, 1995).

Little information are available for using this technique for testing male reproductive performance of ruminants in Egypt (Darwish, 2007). Therefore, the objective of the current study was to evaluate of oral single dose of L-tyrosine (50 g/bull), on semen quality in relation with ultrasonograms pixel intensity and conventional measures of the testes of Friesian bulls.

MATERIALS AND METHODS

The present study was carried out at Sakha Animal Production Research Station, Sakha, Kafe El-Sheikh Governorate, Egypt during the period from September 2008 to January 2010 belonging to the Animal Production Research Institute.

Animals:

Twenty Friesian bulls averaging 8.0±0.24 month of age and 195±15.6 kg body weight were used in this study.

At the beginning of the experiment, the experimental bulls were divided into two similar groups (10 each), according to their LBW and age. Bulls in the 1st group were fed a basal diet and considered as a control group (G1), while those in 2nd group were received an oral dose from L-tyrosine (50 g/bull) at 8.5 months of age. All bulls were judged to be free of physical defects and infected diseases and had normal external genitalia. Animals were kept freely under semi-open sheds and were fed according to the recommendations of Animal Production Research Institute (APRI, 2002) throughout the experimental period (15 months).

Experimental procedures:

Throughout the experimental period, semen was collected from all bulls in each group twice weekly using an artificial vagina from 14 up to 21 month of age. Immediately after collection, the percentages of progressive sperm motility, livability and abnormalities of spermatozoa, as well as, sperm cell concentration were evaluated according to Barth (2002). Different semen characteristics studied were calculated from data collected from 14 to 16 month to obtain mean values of 15 month of age and the period from 19 to 21 month to obtain mean values of 20 month of age.

At 15 and 20 months of age, three ultrasonographic examinations were performed to estimate testicular breadth, and thickness of scrotal layers, seminal cord and testicular mediastinum using a B-mode ultrasound scanner (Ultrascan 900, Alliance Inc., 3173, Louis A. Amos Lachine, Quebec, Canada) connected to a 7.5 MHz linear transducer. The ultrasound setting (focus, gains, brightness and contrast) was standardized. Gel was used as a coupling material between the transducer and the scrotum and minimum pressure was applied to obtain the image. Both testes of each bull were examined by placing the transducer vertically on the caudal aspects of the scrotum. Frozen images included visualization of the mediastinum in order to have an image across the middle of the testis, and the breadth of the testis, then thickness of the mediastinum and breadth were recorded. Ultrasonograms were analyzed with the ultrasound machine using the spot metering technique (Pierson and Adams, 1995) in two 1 cm^3 spots selected approximately 1 cm above the mediastinum and approximately 2 cm from the edge of the image. The mean pixel-intensity (scale: 0-255) from two spots of two tests was calculated.

Then, averages of testicular measurements and pixel intensity value were recorded for each group.

Statistical analysis:

Results were statistically analyzed according to Snedecor and Cochran (1980). A factorial design (2 groups x 2 ages) was used and the statistical model was:

$$Y_{ijk} = U + A_i + B_j + AB_{ij} + e_{ijk}.$$

Where:

Y_{ijk} = Observed values
U = Overall mean
A_i = group
B_j = age
AB_{ij} = Interaction due group x age
e_{ijk} = Random error

The significant differences between means were tested using Duncan Multiple Range Test (Duncan, 1955). Correlation analysis was carried out using computer programme of SAS system (1990). The percentage values of semen characteristics were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages.

RESULTS

Data presented in Table (1) shows that the testicular measurements testicular breadth, and thickness of scrotal layers and seminal cord were not affected significantly by tyrosine treatment. However, testicular breadth was significantly (P<0.01) affected by age, being higher at 20 month than at 15 month of age. Interestingly to note that the effect of interaction between treatment and age on all testicular measures was not significant indicating the highest values in treated bulls at 20 month of age.

Table (1): Conventional testicular measurements of Friesian bulls as affected by L-tyrosine, age and their interaction.

Item	Testicular breadth (mm)	Thickness of scrotal layers (mm)	Thickness of seminal cord (mm)
Effect of Treatment			
L-tyrosine (T)	46.02	4.59	4.87
Control (C)	45.08	4.15	5.13
±SEM	0.49	0.31	0.20
Effect of age			
15 months	43.60[b]	54.35	4.93
20 months	47.50[a]	66.59	5.07
±SEM	0.50	0.31	0.20
Effect of Interaction			
T × 15 month	43.98	4.97	4.78
T × 20 month	48.06	4.22	4.95
C × 15 month	43.23	4.09	5.07
C × 20 month	46.93	4.22	5.20
±SEM	2.1	0.64	0.29

[a] and [b]: Means denoted within the same column (age effect) are significantly (P<0.01) different.

Results presented in Table (2) revealed that L-tyrosine treatment or age did not affect the thickness of testicular mediastinum. However, values of testicular pixel intensity was affected significantly (P<0.01) by treatment and age of bulls, being higher in treated than in control bulls (171.85 *vs.* 100.44) and at 20 than 15 month of age (159.44 *vs.* 112.85). The effect of interaction between treatment and age on ultrasonographic testicular measures was not significant indicating the highest values in treated bulls at 20 month of age.

Data presented in Table (3) shows that bulls treated with L-tyrosine significantly (P<0.01) increased percentages of progressive motility and livability of spermatozoa, and sperm cell concentration compared with the control However, sperm abnormalities significantly (P<0.01) lower in the bulls treated with L-tyrosine (10.97%) than in the control group (20.35%). In general, semen quality of the bulls showed significantly (P<0.01) improved at 20 month as compared to 15 month of age. At 20 month of age, sperm motility and livability, as well as, sperm cell concentration significantly (P<0.01) increased by about 33.25, 22.52 and ٤٣,٨%, while, sperm abnormalities significantly (P<0.01) decreased by 49.4% as compared to those at 15 month of age.

Table (2): Ultrasonographic testicular measurements of Friesian bulls as affected by L-tyrosine, age and their interaction.

Item	Thickness of mediastinum (mm)	Testicular pixel intensity (value)
Effect of Treatment		
L-tyrosine (T)	8.49	171.85[a]
Control (C)	7.17	100.44[b]
SEM	0.60	2.61
Effect of age		
15 months	7.68	112.85[b]
20 months	7.98	159.44[a]
SEM	0.64	2.61
Effect of interaction		
T × 15 month	8.13	145.82
T × 20 moth	8.85	197.88
C × 15 month	6.51	79.88
C × 20 month	7.82	121.00
SEM	0.64	14.61

[a and b]: Means denoted within the same column for each effect are significantly different at P<0.01.

It is worthy noting that the correlation coefficients (CC) between testicular pixel intensity and each of semen characteristics parameters studied were highly significant (P<0.001), being positively the strongest with sperm livability (r= 0.7997), sperm cell concentration (r= 0.7243) and negatively with sperm abnormality (r= -0.7978). However, positively lower correlation was recorded between pixel intensity and sperm motility (r= 0.5493).

Table (3): Physical semen characteristics of bull as affected by tyrosine administration, age and their interaction.

Item	Sperm abnormality (%)	Progressive sperm motility (%)	Sperm livability (%)	Sperm cell concentration ($x10^9$/ml)
Effect of treatment				
L-tyrosine (T)	10.97[b]	73.59[a]	73.79[a]	1.151[a]
Control (C)	20.35[a]	67.27[b]	47.15[b]	0.623[b]
±SEM	1.20	1.82	1.70	0.048
Effect of age				
15 months	18.76[a]	60.39[b]	54.35[b]	0.728[b]
20 months	12.56[b]	80.47[a]	66.59[a]	1.047[a]
±SEM	1.200	1.830	1.700	0.047
Effect of interaction				
T × 15 month	13.35	62.82	66.59	0.979
T × 20 month	8.59	84.35	81.00	1.324
C × 15 month	24.18	57.95	42.12	0.477
C × 20 month	16.53	76.59	52.18	0.769
±SEM	1.67	9.540	9.250	0.067
Correlation (r) with pixel intensity	-0.7978***	0.5493***	0.7997***	0.7243***

[a and b]: Means denoted within the same column for each effect are significantly different at P<0.01. *** Significant at P<0.001

DISCUSSION

The current study aimed to evaluate the oral treatment with L-tyrosine on semen quality in relation with ultrasonograms pixel intensity and conventional measurements of the testes of Friesian bulls. Regard to the results of conventional testicular measurements including testicular breadth, and thickness of scrotal layers and seminal cord (Table 1), only testicular breadth was affected significantly ($P<0.01$) by age, being higher with age advancing from 15 than 20 months of age. Such trend was naturally associated with the testicular growth and development in relation with animal growth rate. Testicular volume, as estimated by measuring scrotal circumference or testicular size, is often used to predict sperm production (Lunstra *et al.*, 1985 and Toelle and Robison 1985).

Our results indicated that thickness of testicular mediastinum was not affected by L-tyrosine treatment or age. Similarly, thickness of testicular mediastinum in bull with normal semen did not differ significantly from those with poor semen quality (Eilts and Peachman, 1988 and Darwish, 2007). However, many authors (Gouletsou *et al.*, 2003; Abdel-Razek and Ali, 2005) reported the opposite.

It is of interest to note that, the trend of increase in testicular breadth with age progress was not associated with testicular ultrasonic measurements (thickness of mediastinum). These results are in agreement with those reported by Cartee *et al.* (1989), who found no correlation between scrotal circumference and ultrasonic measurements of testicles. However, Rocha *et al.* (1990) found that ultrasonographic assessment of testicular diameter was more highly correlated with testicular volume than with scrotal circumference. Also, Lenz *et al.* (1994) reported that ultrasonographic measurements of testicular volume in men were correlated ($P<0.001$) with sperm count. Computerized analysis of ultrasonographic images can provide substantial information regarding the structure and function of tissues (Pierson and Adams, 1995).

Numerical values of testicular pixel intensity significantly ($P<0.01$) increased by treatment and advancing age of bulls. In agreement with the present results, Gabor *et al.* (1998) indicated that testicular pixel intensity has considerable potential for evaluation of testicular function in bulls. In this respect, Powe *et al.* (1988) detected an abnormal appearance of the testicular parenchyma in pathological states by ultrasonography in bulls. The image of the parenchyma of the testis in bulls has a homogenous appearance with moderate echoicity (Eilts and Pechman, 1988). Ultrasonography holds promise for the prediction of breeding capability in the mature bull (Arauindakshan *et al.*, 2000). Testicular ultrasonographic echotexture has considerable promise for augmenting breeding soundness examination of bulls (Kastelic *et al.*, 2001) and is considered as another method for assessing spermatogenetic capacity (Gabor *et al.*, 1998). So, the advent of more sophisticated ultrasound equipment and techniques in image analysis will likely improve the ability to predict semen quality by evaluation of testicular ultrasonograms (Andres *et al.* 2005). The observed increase

(P<0.01) in numerical values of testicular pixel intensity between 15 and 22 month of age in this study is in agreement with Crim and Geschwind (1972); Lafortune *et al.* (1984) and Monet-Kuntz, *et al.* (1984), who mentioned that the increase in numerical pixel values from 8 to 22 week of age could have been due to the gradual formation of the more mature cell types of spermatogenesis

In our study, all semen characteristics studied significantly (P<0.01) improved in bulls treated with L-tyrosine and by advancing age. These findings were associated with increasing numerical values of testicular pixel intensity significantly (P<0.01) by about 71% in tyrosine group (improved semen) than that in control group (normal semen) and higher by about 42% at 20 than at 15 month of age. These findings were indicated also by strong and significant correlation between numerical values of testicular pixel intensity and each of semen characteristics studied (Table 3). This may indicate strong relationship between testicular pixel intensity and density of seminiferous tubule area and in turn semen quality. Arteaga *et al.* (2005) found that testicular pixel intensity increased coincidently with increasing semen quality. Also, Kastalic *et al.* (2001) found that the testicular pixel intensity values were in conjunction with the percentages of motile and morphologically normal sperm in Angus cross bulls. Furthermore, Gabor and Szasz (1989) suggested good associations between testicular pixel intensity and semen characteristics of bulls. Therefore, Gabor and Szasz (1989) suggested that the testicular pixel intensity might be used to predict semen quality.

It is of interest to note that treatment of L-tyrosine improved (P<0.05) semen quality as compared to control. The present results come in agreement with the findings of El-Amrawi, (1997) studied the effect of L-tyrosine on fertility of bull and buffalo-bull. He found that, a single oral dose of L-tyrosine leads to improvement in testosterone level, semen quality and fertilizing capacity in both buffalo-bull and bulls.

In conclusion, an oral single dose of L-tyrosine (50 g/bull) showed beneficial effects on semen quality of Friesian bulls. The testicular ultrasonograms pixel intensity may be used as a good tool for prediction of semen quality at early ages. Also, such technique may be useful for determining other testicular measurements such as testicular breadth, and thickness of scrotal layers and seminal cord.

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استخدام الموجات فوق الصوتية في مقاييس الخصية وعلاقتـه بجـودة الـسائل المنوي لطلائق الفريزيان المعاملة بالتيروزين.

محمد عوض أبو الحمد و شريف مغاورى شامية
معهد بحوث الإنتاج الحيواني، مركز البحوث الزراعية، وزارة الزراعة، دقـى، جيـزة، مـصر.

تهدف هذه الدراسة إلي تقييم استخدام الموجات فوق الصوتية وكثافة مكونات الصورة (بيكسل) لقياس بعض مقاييس الخصية وعلاقة ذلك بجودة السائل المنوي للطلائق الفريزيان المعاملة بجرعة واحدة عن طريق الفم من التيروزين (٥٠ جم /طلوقة)، على خصائص السائل المنوي وعلاقة مع كثافة البكسل ومقاييس الخصية في العجول الفريزيان. واستخدمت ٢٠ طلائق فريزيان (متوسط أعمرها ٨,٠ ± ٠,٢٤ شهر ووزن ١٩٥ ± ١٥,٦ كجم). وقسمت إلى مجموعتين تجريبيتين متساويتين ، ١٠ طلائق في كل مجموعة. غذيت الطلائق في المجموعة الأولي علي العليقة الأساسية (الكنترول)، في حين أن المجموعة الثانية تم تجريعها عن طريق الفم جرعة واحدة من التيروزين (٥٠ جم / طلوقة) عند عمر ٨,٥ شهر. وخلال الفترة التجريبية تم جمع السائل المنوي لتقييم النسبة المئوية الحركة التقدمية والحيوانات المنوية الشاذة ، وكذلك تركيز الحيوانات المنوية. وعند عمر ١٥ و ٢٠ شهر من العمر تم قياس عرض الخصية ، وسمك جلد كيس الصفن والحبل المنوي ، وسمك طبقة mediastinum الخصية و قيمة كثافة البيكسل باستخدام الموجات فوق الصوتية. وأظهرت النتائج أن عرض الخصية وسمك جلد كيس الصفن والحبل المنوى للخصية لم تتأثر بشكل معنوي بالمعاملة بالتيروزين. ومع ذلك ، كان عرض الخصية أعلى عند عمر ٢٠ عن ١٥ شهر من العمر. ولم يأثر المعاملة التيروزين أوالعمر على عرض الخصية. ومع ذلك ، كان قيم كثافة بيكسل أعلى للخصية ($P<0.01$) في الطلائق المعاملة بالمقارنة بالكنترول وعند عمر ٢٠ شهر عن ١٥ شهر من العمر. وأظهرت المعاملة بالتيروزين إلي زيادة معنوية ($P<0.01$) نسبة الحركة وتركيز الحيوانات المنوية وانخفاض معنوي ($P<0.01$) في النسبة للحيوانات المنوية الشاذة بالمقارنة مع الكنترول. وعند عمر ٢٠ شهر زادت قدرة الحيوانات المنوية على الحركة ونسبة الحي فضلا عن تركيز الحيوانات المنوية زيادة معنوية ($P<0.01$) بنحو ٣٣,٢٥ ، ٢٢,٥٢ و ٤٣,٨٪ وانخفض نسبة الحيوانات المنوية الشاذة ($P<٠,٠١$) بمعدل ٤٩,٤ ٪ بالمقارنة مع تلك الموجودة في ١٥ شهر من العمر. وكان معامل الارتباط بين كثافة البكسل للخصية وكل من صفات السائل المنوي المدروسة هام جدا ($P<٠,٠٠١$) ، وظهر بشكل إيجابي قوى مع الحيوانات المنوية الحية (ر = ٠,٧٩٧٨) ، تركيز الحيوانات المنوية (ر = ٠,٧٢٤٣). وسلبا مع الحيوانات المنوية الشاذة (ر = -٠,٧٩٧٨) ، ومع ذلك تم تسجل أدنى ارتباط إيجابي بين كثافة بكسل وحيوية الحيوانات المنوية (ر = ٠,٥٤٩٣).

قام بتحكيم البحث

أ.د / عبد الخالق السيد عبد الخالق	كلية الزراعة – جامعة المنصورة
أ.د / علاء السيد زيدان	مركز البحوث الزراعية