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TESTICULAR ULTRASONOGRAMS PIXEL INTENSITY AS INDICATOR FOR SEMEN QUALITY OF BUFFALO BULLS

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ABSTRACT

This study aimed to determine the relationship between semen quality and testicular pixel intensity derived from image analysis of ultrasonograms in buffalo bulls and the possibility of using this technique to predict semen quality.

Fourty buffalo bulls averaging 30 months of age and 505 kg body weight were used in this study. Semen was collected and evaluated (motility, abnormality and concentration) twice weekly and testicular ultrasonographic examinations were done 3 times during the experimental period (12 weeks) with six weeks interval to determine some of testicular measures (Medisatinum width, testicular breadth and pixel intensity). Bulls were allocated into two groups according to results of semen evaluation. The 1st group (n=19) had normal semen quality and the 2nd one (n=21) had poor semen quality.

Results show that both mediastinum width and scrotal circumference did not differ significantly in normal and poor semen groups. However, values of ultrasound testicular breadth and testicular pixel intensity was significantly ($P<0.05$) higher by 8.2 and 71%, respectively in normal group than in poor semen group. Values of sensitivity and specificity of testicular pixel intensity were higher for future semen than that for the current semen (present semen collected), but the difference was significant ($P<0.05$) only for speicificy. Values of negative predictive were higher than the positive predictive for both current and future semen.

In conclusion, the testicular ultrasonogram pixel intensity may be use as a good tool for prediction of semen quality in future for buffalo bulls at early ages. So, such technique may be useful for early selecting and discarding bulls with unsatisfactory semen quality, which reflects economically on bull management.

Keywords: Buffalo bulls, Ultrasonographic, semen, testicular pixel intensity.

INTRODUCTION

Fertility is a fragile parameter that may be varying temporarily or be permanently depressed. Abnormalities of the testis may directly affect the bull's ability or may alter the quality of bull semen (Van Camp, 1997). In large buffalo herds, incidence of a considerably large number of bulls with poor semen is apparent (Ahmed *et al.*, 1988). The poor quality semen of buffalo bulls was associated with abnormalities in anatomy and histology of the testis (Ahmed *et al.*, 1988 and Van Camp, 1997).

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.

Recently, the 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). Using this technique is well described for reproductive status of female cattle (Pirson *et*

al., 1988), but few information is available for using this technique for testing male reproductive performance of ruminants.

The testicular transcutaneous ultrasound images may provide more useful information than measuring scrotal circumference in bull (Pechman and Eilts, 1987). The image of the parenchyma of the testis in bulls has an homogenous appearance with moderate echoicity (Eilts and Pechman, 1988). In bulls, Powe *et al.* (1988) detected an abnormal appearance of the testicular parenchyma in pathological states by ultrasonography.

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 (Griffin and Ginther, 1992). 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). Recently, the use of computer assisted analysis has add new power to reproductive ultrasonography. This analysis gives pixel,each pixel represents a discreet tissue reflectors as it relates to the reflection of the ultrasound beams and is represented by one of 256 shades of grey ranging from 0 (black) to 255 (white). This numerical pixel values increase analysis value of ultrasnographic images and can provide substantial information regarding the function and structure of tissue (Pierson and Adms, 1995).

Ultrasonography holds promise for the prediction of breeding capability in the mature bull (Aravindakshan *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).

Therefore, the objective of the current study was to evaluate the relationship between semen quality and pixel intensity of testicular ultrasonograms. Also, the ability to predict semen quality based on testicular pixel intensity was included in this study.

MATERIALS AND METHODS

Animals:

Fourty buffalo bulls ranging from 24-36 months of age and from 450-550 kg body weight belonged Mehallet Mousa Research Station, Animal Production Research Institute were used in this study for an experimental period of 12 weeks. All bulls were free of physical defects and infected diseases and had normal external genitalia. Animals were kept freely under semi-opened asbestos sheds and were fed according to the recommendations of Animal Production Research Institute (APRI, 2002).

Experimental procedures:

Throughout the experimental period of 12 weeks, semen was collected from all bulls twice weekly using an artificial vagina. Percentage of motility and abnormality of spermatozoa as well as sperm concentration was determined in collected ejaculates according to Barth (2002). Scrotum

circumference was measured by tap while, three ultrasonographic examinations were performed at zero time, 6 weeks and 12 weeks (6-week interval) to estimate thickness of the testicular mediastinum and testicular breadth with 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. Images were recorded with a super VHS videocassette recorder for later analysis. Images were acquired from the videotape as a 640 x 480 pixel image using a personal computer. Grey scale values of the testicular parenchyma of each testis were determined, 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 and expressed in pixel intensity units by using software developed for this purpose. The mean pixel intensity for each bull was calculated.

Bulls were divided into two groups according to the evaluated semen characteristics (Hafez and Hafez, 2000). According the performed evaluation, 19 bulls had semen of satisfactory quality (≥60% sperm motility, ≥70% morphologically sperm normality and sperm concentration of ≥0.5 x 10^9/ml) and were served as normal semen group. However, twenty one bulls had less than the above values (unsatisfactory quality) and were considered as poor semen group. Then, averages of testicular measures, pixel intensity value were recorded for each group.

The cut-off value of pixel intensity used to predicting good quality semen was 105, the least difference between sensitivity and specificity of pixel intensity was obtained at this cut-off value. Values of pixel intensity for predicting semen quality were calculated according to (Martin *et al.*, 1987) as follows:

The sensitivity (a/a + d x 100)
The specificity (c/c + b x 100)
The positive predictive value (a/a + b x 100)
The negative predictive value (c/c + d x 100)

Where:

(a) Correct positive diagnosis
(b) Incorrect positive diagnosis
(c) Correct negative diagnosis
(d) Incorrect negative diagnosis

Statistical analysis:

Data were analyzed using SAS (1999), GLM. Analysis of variance was used to compare the testicular measures of between normal and poor semen groups. Chi-square was used to compare the sensitivity, specificity and predictive value of present and future semen quality.

RESULTS AND DISCUSSION

Regard to the results of testicular measures shown in table (1), thickness of testicular mediastinum did not differ significantly in normal group than in poor semen group. This finding agreed with that reported by Eilts and Pechman (1988), however, both Gouletsou *et al.* (2003) and Abdel-Razek and Ali (2005) found a contrasted trend of that reported in this study, this may be attributed to age and breed differences.

On the other hand, values of ultrasound testicular breadth was higher by 8.2% in the normal semen group than in poor semen group. Also, there were significant differences between both groups in testicular breadth.

It is of interest to note that scrotal circumference was not associated with testicular ultrasonic measurements (thickness of mediastinum and testicular breadth, Fig. 1). In spite of the significant differences between both groups in breadth, scrotal circumference did not differ significantly between normal and poor semen groups (Table 1).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. Also, Joachim *et al.* (1989) reported that the ultrasonic measurements of the testis were not correlated significantly with semen quality.

Table (1): Testicular measures of buffalo bulls in normal and poor semen groups.

Testicular measure	Normal semen group	Poor semen group	Sig.
Thickness of mediastinum (cm)	0.51±0.004	0.50±0.003	NS
Testicular breadth (cm)	5.78±0.05	5.34±0.06	*
Scrotal circumference (cm)	32.7±0.08	32.8±0.09	NS
Testicular pixel intensity (unit)	177.6±3.16	104.0±1.81	**

NS = Not significant * P<0.05 ** P<0.001

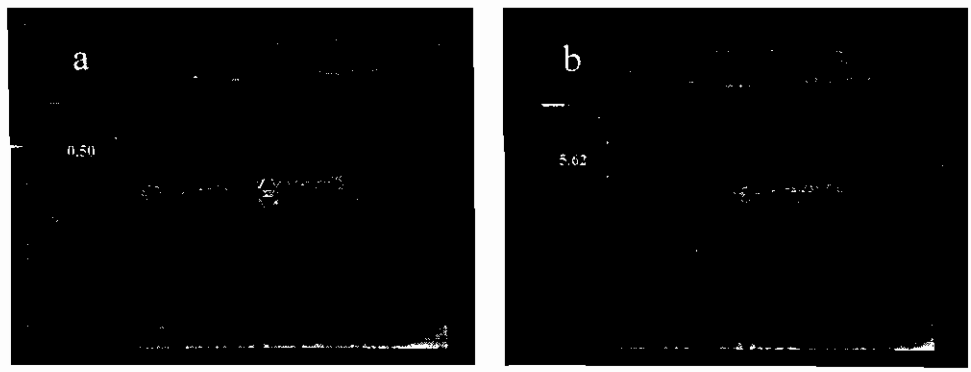

Fig. (1): Ultrasonic of the testicular mediastinum thickness (a) and testicular breadth (b).

Concerning the testicular pixel intensity, it was significantly (P<0.01) higher by about 71% in normal group than that in poor semen group(Table 1).

This may indicate a strong correlation between testicular pixel intensity and density of seminiferous tubule area and in terms of semen quality. The relationship between testicular histometry and semen quality was identified by Abdel-Khalek *et al.* (2001), who found that density and proportional area of the seminiferous tubules were significantly higher in sub-fertile than in fertile buffalo 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.

The obtained significant difference in testicular pixel intensity, being higher in normal than poor semen group agreed with Arteaga *et al.* (2005), who found that testicular pixel intensity decreased coincidently with decreasing semen quality. Furthermore, Gabor and Szasz (1999) suggested good associations between testicular pixel intensity and semen characteristics of Holstein Friesian and Austrian Fleckvieh bulls.

The mean value of pixel intensity for normal semen quality group was higher than that in poor semen quality group in different experimental period (Table 2). Also, the pixel intensity values were decreased with decreasing semen quality. This findings were agree with Kastelic *et al.* (2001) who found that testicular pixel intensity values were in conjunction with the percentage of motile and morphologically normal sperm in Angus cross bulls.

Table (2): Quality of normal and poor semen and testicular pixel value for bulls in different of experimental period.

Experimental Period	Group	Semen quality		Testicular Pixel intensity	
				Mean ± SE	Range
Zero time	Normal semen group	Prog. Motility, %	78.1±4.2	187.9±3.40	166-210
		Normal morph, %	84.2±3.2		
		Sperm conc. ($x10^9$)	0.91±0.04		
	Poor semen group	Prog. Motility, %	49.3±3.8	109.0±2.10	92-124
		Normal morph, %	60.0±4.5		
		Sperm conc. ($x10^9$)	0.50±0.04		
6 weeks	Normal semen group	Prog. Motility, %	73.3±3.5	178.6±3.1	160-200
		Normal morph, %	80.0±4.1		
		Sperm conc. ($x10^9$)	0.89±0.05		
	Poor semen group	Prog. Motility, %	50.0±3.3	105.3±1.98	96-122
		Normal morph, %	59.9±4.1		
		Sperm conc. ($x10^9$)	0.41±0.03		
12 weeks	Normal semen group	Prog. Motility, %	79.8±4.3	167.9±2.9	160-195
		Normal morph, %	86.0±4.3		
		Sperm conc. ($x10^9$)	0.85±0.03		
	Poor semen group	Prog. Motility, %	47.0±4.6	98.0±1.77	96-120
		Normal morph, %	55.1±6.2		
		Sperm conc. ($x10^9$)	0.40±0.04		

The results presented in Table (3) show that values of sensitivity and specificity of testicular pixel intensity were higher for future semen than that for the current semen. However, the differences were only significant ($P<0.05$) for specificity between current and future semen.

These results were supported by Arteaga *et al.* (2005), who found that the evaluation of testicular pixel intensity was more likely associated with the future semen than with the current semen quality. Generally, Gabor and Szasz (1999) suggested that the testicular pixel intensity might be used to predict semen quality and /or non return rate.

Table (3):Sensitivity (%), specificity (%) and predictive value of testicular ultrasonogram pixel intensity to predict semen quality of buffalo bulls at current and future collected semen.

Item	Current Semen	Future semen	Sig.
Correct positive diagnosis (a)	9	8	-
Incorrect positive diagnosis (b)	10	11	-
Correct negative diagnosis (c)	17	18	-
Incorrect negative diagnosis (d)	4	3	-
Sensitivity %	69.2	72.7	NS
Specificity (%)	30.0	61.1	*
Positive predictive value	47.4	42.1	NS
Negative predictive value	81.0	85.7	NS

NS = Not significant * P<0.05

The present values of negative predictive were higher than the positive predictive for both current and/or future semen (Table 2). This findings come in line with that reported by Joachim *et al.* (1989), who reported that the ultrasound was of value in examining the testes of bulls with low sperm output and poor semen quality.

In conclusion, the testicular ultrasonogram pixel intensity may be use as a good tool for prediction of semen quality in future for buffalo bull at early ages. So, such technique may be useful for early selecting and discarding bulls with unsatisfactory semen quality, which reflects economically on bull management.

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قياس الكثافة الضوئية للنسيج الخصوى بجهاز الموجات فوق الصوتية كدلالة على جودة السائل المنوى للطلائق الجاموسى

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تهدف هذه الدراسة الى تقدير العلاقة بين صفات السائل المنوى والكثافة الضوئية للنسيج الخصوى من خلال تحليل صور جهاز الموجات فوق الصوتية للخصيتين لطلائق الجاموسى وامكانية استخدام هذه التقنية للتنبؤ بصفات السائل المنوى .

استخدم فى هذه الدراسة عدد ٤٠ طلوقة جاموسى تراوح عمرها بين ٢٤-٣٦ شهر وتراوح وزنها بين ٥٠٠-٥٥٠ كجم ، تم جمع السائل المنوى لهذه الطلائق مرتين اسبوعيا وتم تقييم صفات السائل المنوى لها ، كما تم فحص الخصيتين باستخدام جهاز الموجات فوق الصوتية ثلاث مرات (مرة كل ستة اسابيع) خلال الفترة التجريبية (١٢ اسبوع) لاخذ بعض القياسات (Testicular breadth -Mediastinum thickness-Pixel intensity). قسمت الطلائق الى مجموعتين طبقا لتقييم صفات السائل المنوى ، المجموعة الاولى (١٩ طلوقة) كانت ذات صفات سائل منوى طبيعية ، بينما كانت المجموعة الثانية (٢١ طلوقة) ذات صفات سائل منوى لها رديئة.

أوضحت النتائج عدم وجود اختلاف معنوى بين المجموعتين بالنسبة لقياس Mediastinum width ومحيط كيس الصفن ، بينما كانت الاختلافات معنوية عند مستوى (٠,٠٥%) بين المجموعة ذات صفات السائل المنوى الطبيعية بالنسبة لقياس testicular breadth and pixel intensity اعلى بمعدل ٨,٢ ، ٧١% عن المجموعة رديئة صفات السائل المنوى للقياسين على التوالى. كانت قيم الـ sensitivity and specificity of testicular للكثافة الضوئية للنسيج الخصوى عالية التنبؤ بصفات السائل المنوى المستقبلى (بعد ٦ اسابيع من فحص السونار) عن صفات السائل المنوى الموجود فعلا (يوم فحص السونار) وكانت الفروق معنوية (٠,٠٥%) فقط بالنسبة للـ specificity. كانت قيم الـ Negative predictive اعلى من الـ Positive predictive فى كل من صفات السائل المنوى المستقبلى عن صفات السائل المنوى الموجود فعلا.

نستخلص من هذه الدراسة ان تقنية قياس الكثافة الضوئية للنسيج الخصوى بجهاز الموجات فوق الصوتية يمكن استخدامها كتقنية جيدة للتنبؤ بصفات السائل المنوى للطلائق الجاموسى مستقبلا فى عمر مبكر ومثل هذه التقنية تفيد فى الاستبعاد المبكر والانتخاب للطلائق ذات صفات السائل المنوى الردئية لما لها من مردود اقتصادى فى رعاية الطلائق.