

RELATIONSHIPS BETWEEN THE MORPHOMETRIC AND HISTOMETRIC CHARACTERISTICS OF THE ACCESSORY SEX GLANDS AND SEMEN VOLUME OF EGYPTIAN BUFFALO BULLS

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ABSTRACT

Morpho-histometric characteristics of the accessory sex glands were studied in eight buffalo bulls, four sub-fertile bulls having low semen ejaculate volume (G1) and four mature buffalo bulls with normal semen ejaculate volume (G2). Bulls in both groups were of similar age and live body weight. All bulls were from the same herd and fed and managed alike. Results revealed that the low ejaculate volume in G1 was associated significantly with low sperm motility percentage ($P < 0.001$), sperm concentration ($P < 0.05$) and testosterone concentration in blood plasma than those in G2. Weight, length, and width of the seminal vesicles were significantly ($P < 0.05$) greater in G2 than in G1 by about 62, 54 and 59%, respectively. However, all measures of the prostate and Cowper's glands did not differ significantly between both groups. Significantly positive correlation coefficients were found between ejaculate volume and weight ($r = 0.94$, $P < 0.001$), length, ($r = 0.58$, $P < 0.01$) or width ($r = 0.38$, $P < 0.05$) of the seminal vesicles. Histometric data including thickness of tunica musculosa and interlobular septa, and diameter of alveoli were significantly ($P < 0.05$) higher in the vesicular glands of G2 than that of G1 by about 60, 36 and 36 %, respectively. While, thickness of inter-alveolar stroma was significantly ($P < 0.05$) higher in G1 than in G2 (32 vs. 28 μm). In pars interna of the prostate gland, thickness of interlobular trabecula was significantly ($P < 0.05$) higher in G1 than in G2 by about 16%, however, diameter of secretory acini significantly ($P < 0.05$) decreased in G1 than in G2 (47.2 vs. 55.5 μm).

The present study proved a strong relationship between morpho-histometry of the seminal vesicles and histometry of the prostate gland on one side, and ejaculate semen volume of Egyptian buffalo bulls on the other side. Ejaculate semen volume was not affected the morpho-histometric characteristics of Cowper's glands.

Keywords: Buffaloes, semen, morpho-histometry, seminal vesicles, prostate.

INTRODUCTION

Ejaculate volume is an important measurement of semen quality characteristics, especially for preparing doses of AI threshold level (Dan Dass, 1992). The seminal vesicles and prostate secretions are mainly responsible for ejaculate volume and contents of protein, fat and carbohydrate in seminal plasma affecting viability of the semen (Sudhakar *et al.*, 1985). Abnormalities in the accessory sex organs directly affect semen quality, especially ejaculate volume. Seminal vesiculitis being the most common disease in the infertile bulls (Ahmed *et al.*, 1988 and Van Camp, 1997).

Several studies were carried out on morphological and anatomical development of the accessory sex glands in normal Egyptian buffalo bulls

(Darwish, 1996 and Osman, 1996), while few results were reported on histogenesis of the accessory glands of Egyptian buffalo bulls as affected by fertility (Aboul Omran, 2005) as well as seminal vesicles (Sudhakar *et al.*, 1986) and prostate gland (Sudhakar *et al.*, 1985) of Murrah buffalo bulls.

In Egyptian buffalo bulls, no available information were found concerning histometry of the accessory sex glands in relation to ejaculate volume. Therefore, the current study aimed at providing knowledge on morpho-histometry of the seminal vesicles, prostate gland and Cowper's glands of Egyptian buffalo bulls, fertile having normal semen volume compared with sub-fertile bulls having low semen volume.

MATERILS AND METHODS

The experimental work included four buffalo bulls culled for their low semen volume averaging 38.9 ± 5.2 mo of age and weighing 486.6 ± 42 kg LBW (G1) compared to four normal bulls aging 37.7 ± 4.9 mo with 491.3 ± 46 kg LBW (G2). All bulls were taken from the buffalo herd of Mehallet Mousa Experimental Station belonging to Animal Production Research Institute, Agricultural Research Center.

All bulls were free of infectious diseases having healthy appearance appropriate for semen production. Bulls in both groups were subjected to the same system of feeding and management.

Semen was collected from all bulls twice weekly using an artificial vagina over a collection period of 12 weeks. Bulls were sexually stimulated by allowance of 2-3 false mounts prior to complete ejaculation. Semen volume, sperm cell concentration and percentage of sperm motility were determined in the fresh semen of the second ejaculate every collection day.

At the end of the collection period, blood samples were taken from the jugular vein of each bull before slaughtering to determine testosterone concentration using active RIA (Diagnostic System Lab., Inc. USA).

Preslaughter weights of all bulls were recorded and immediately after slaughtering, the genital tract was dissected out of the carcass and trimmed from the attached fat. Genital sex glands including seminal vesicles, prostate gland and Cowper's glands were weighed and their lengths were estimated, whereas, circumference of prostate gland was considered as a length. However, their dimensions (width and thickness) were measured at different locations along their length.

Fresh specimens were immediately taken from the middle portion of each lobe of the seminal vesicles (Sudhakar *et al.*, 1986). Also, specimens of prostate gland were taken ventrally from the middle portion of the pelvic urethra, since the distribution of pars interna is comparatively more in the lateral and ventral walls than in the dorsal wall as described by Sudhakar *et al.* (1985).

All specimens were immediately fixed in buffered 10% neutral formol and were transported to the Physiology Laboratory, Department of Animal Production, Faculty of Agriculture, Mansoura University. After a fixation period of 24-48 hours, all specimens were processed for routine paraffin

technique. Paraffin sections (8-10 μ m) were stained by Harris Haematoxyline and Eosin according to Drury and Wallington (1980).

In each group, a total number of 40 sections was examined in five microscopic fields of each seminal vesicles lobe (5 fields x 2 lobes x 4 animals). In addition, 20 sections of pars interna of the prostate gland (5 fields x 4 animals) were examined histologically using research microscopy and histometrically by means of eyepiece in micrometer.

Data were statistically analyzed by using T-test according to Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

Semen quality:

Ejaculate volume of semen collected from bulls of both groups through the collection weeks (Table 1) was consistently and significantly ($P < 0.01$) higher in bulls of G2 than in bulls of G1 (Fig. 1). The overall mean of the ejaculate volume in G1 was only 38% of that in G2 (100%) and the difference was significant ($P < 0.001$).

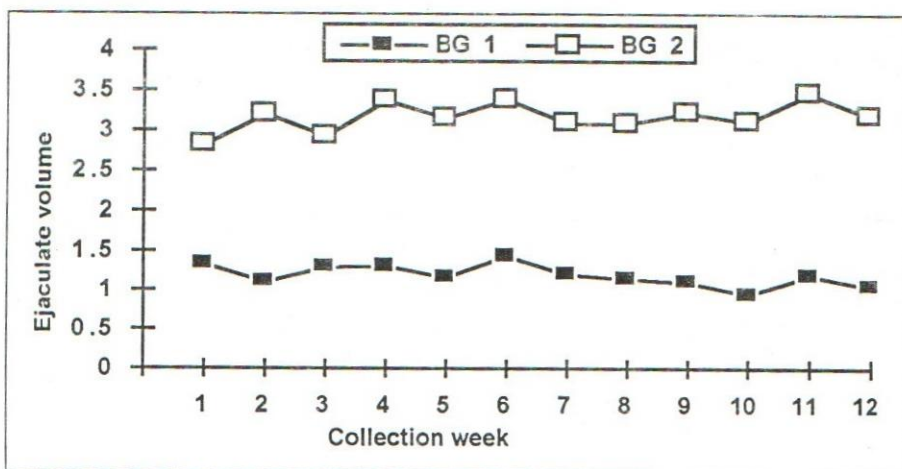


Figure (1): Semen ejaculate volume of culled (G1) and normal (G2) bulls through collection weeks.

On the basis of the overall means during the collection period, the lower ejaculate volume was almost associated with significantly lower sperm motility percentage ($P < 0.001$) and sperm cell concentration ($P < 0.05$, Table 1)

Table (1): Semen characteristics of the experimental bulls.

Characteristic	G1	G2	Sign.
Ejaculate volume (ml)	1.19 \pm 0.13	3.17 \pm 0.18	***
Sperm motility (%)	62.8 \pm 3.7	84.2 \pm 1.7	**
Sperm cell concentration ($\times 10^9$ /ml)	0.868 \pm 0.12	1.128 \pm 0.02	*

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Blood testosterone profile:

Testosterone concentration in peripheral blood plasma was significantly ($P < 0.01$) higher in G2 than in G1 by 46.5% (0.705 vs. 0.481 ng/ml), indicating a strong relationship between semen volume and testosterone concentration in buffalo bulls as was reported in Friesian (Abdel-Khalek, 1999) and Egyptian buffalo bulls (Darwish, *et al.*, 2001). In the present study, the correlation coefficient of testosterone level was significantly ($P < 0.001$) positive with ejaculate volume ($r = 0.82$), weight of the seminal vesicles ($r = 0.71$) and prostate gland ($r = 0.68$). Darwish (1996) and El-Siefy (1999) recorded similar correlations in Egyptian buffalo bulls.

Anatomical study:

The morphological measures of the accessory sex glands presented in table (2) show that weight, length and width of the seminal vesicles were significantly different between both bull groups. Seminal vesicles of G2 were significantly heavier ($P < 0.01$), longer, and wider ($P < 0.05$) than those of G1 by about 62, 54, and 59%, respectively. The present values of the seminal vesicles in the normal bulls (G2) are nearly similar to those reported in sexually mature Egyptian buffalo bulls (Osman, 1965), while in G1 values are in agreement with those reported in bulls aged 24 mo (Osman, 1996). The differences in such measurements of either prostate or Cowper's glands between G1 and G2 were not significant, however, their values tended to be higher in G2 (Table 2).

Table (2): Weight and dimensions of the accessory sex glands of the experimental bulls.

Measurement	G1	G2	Sign.	Correlation with ejaculate volume
Seminal vesicles				
Weight (g) ¹	9.2±1.90	14.9±1.8	**	0.94 ^{***}
Length (cm)	5.7±0.29	8.8±0.72	*	0.58 ^{**}
Width (cm)	1.7±0.18	2.7±0.36	*	0.38 [*]
Thickness (cm)	0.68±0.14	0.78±0.24	NS	0.006
Prostate gland				
Weight (g)	4.10±0.55	4.50±0.45	NS	0.30
Length (cm)	2.21±0.08	2.25±0.10	NS	-0.05
Width (cm)	1.50±0.43	1.90±0.27	NS	0.36
Thickness (cm)	0.79±0.09	0.83±0.04	NS	0.24
Cowper's glands				
Weight (g)	7.58±0.83	9.58±1.0	NS	0.22
Length (cm)	4.2±0.68	3.90±0.41	NS	0.02
Width (cm)	1.40±0.10	1.63±0.27	NS	0.06
Thickness (cm)	0.70±0.12	0.95±0.16	NS	0.21

NS = Not significant * $P < 0.05$ ** $P < 0.01$ *** $P > 0.001$

¹ = Average weight of right and left side

It is of interest to note that results of correlation (Table 2) were in the same trend, whereas, measurements of seminal vesicles showed significantly positive correlation with ejaculate volume. The highest correlation coefficient was recorded between ejaculate volume and seminal vesicles weight ($r= 0.94$). Darwish, (1996) found that the correlation coefficient of LBW with weight of the seminal vesicles was significantly higher ($r= 0.83$) than that with weight of the prostate ($r= 0.68$). Hence, the nearly similarity of LBW in both bull groups, and the significantly higher weight and dimensions of the seminal vesicles in G2 than G1 may indicate undeveloped seminal vesicles in G1. Ahmed *et al.* (1985 & 1988) reported that the poor quality semen of the low-fertile buffalo bulls was almost associated with undeveloped accessory sex glands and bulls showed seminal vesiculitis.

The present morphometric data may reflect the effective role of the seminal vesicles, especially their weight, on the seminal plasma production. The seminal vesicles participate in production of more than 50% of the seminal plasma (Hafez and Hafez 2000). Volume of each semen ejaculate is an important measurement of semen physical characteristics, particularly for preparing artificial insemination doses at threshold level (Dan Dass, 1992).

Histological study:

Seminal vesicles:

The seminal vesicles examined in all bulls were morphologically lobulated and composed of tubulo-alveolar glands. Each lobe was histologically composed of three main layers, tunica adventitia, tunica musculosa and tunica mucosa. Similar structure was described in Murrah buffalo bulls (Sudhakar *et al.*, 1986) and in other domestic animals (Dellmann and Wrobel, 1976).

The covered tunica adventitia in all bulls consisted of a loose connective tissue containing different types of fibers and many blood vessels. This finding is in agreement with that observed in Murrah buffalo bulls (Sudhakar *et al.*, 1986), goats (Gupta, 1978) and man (Glenister, 1976). Generally, thickness of tunica adventitia did not differ significantly between both bull groups (Table 3 and Fig. 1).

In all bulls, tunica musculosa was observed to surround each vesicular lobe consisting of smooth muscle fibers arranged almost in circular type in G2 and in circular and longitudinal types in 50% of bulls of G1. Sudhakar *et al.* (1986) described the two types of tunica musculosa in Murrah buffalo bulls up to two years of age and only circular type in sexually mature bulls. Similar types were observed in goats as affected by age (Gupta, 1978). In the present study, thickness of tunica musculosa significantly ($P<0.01$) increased by about 60% in G2 than in G1 (Table 3).

Septa of muscular connective tissue originated from tunica musculosa were seen to divide each vesicular lobe into small lobules consisting interlobular septa. These septa contained fibroblasts, smooth muscle fibers, different types of fibers and blood vessels. Average thickness of the interlobular septa was significantly ($P<0.05$) higher in G2 than G1 (Table 3).

In all bulls tunica mucosa was folded and consisted of two laminae, propria and epithelialis. The propria was composed of a loose connective tissue including alveoli, inter-alveolar tubules and intralobular ducts, forming inter-alveolar stroma. The vesicular alveoli were compact and the inter-alveolar stroma significantly ($P>0.05$) decreased in G2 compared to G1 (Table 3 and Figs. 2 and 3).

Within the lamina propria, there were vesicular alveoli lined with a single layer of pseudo-stratified columnar epithelial cells (Fig.4 and 5). Similar findings were observed in Murrah buffalo bulls (Sudhakar *et al.*, 1986), bovine bulls (Trotter, 1959) and goats (Gupta, 1978). At comparing the alveolar system in both groups, it was found that average diameter of alveoli significantly ($P<0.05$) increased in G2 than G1 (129 vs. 95 μm). However, the duct system did not differ significantly between both groups in terms of average diameter of inter-alveolar tubules and intralobular ducts (Table 3).

The previous results concerning the histometry of the seminal vesicles reflects the well developed alveolar system consequently the higher secretory activity of bulls in G2 than in G1, indicating the role of the seminal vesicles in production of seminal plasma

Table (3): Histometry of seminal vesicles of experimental bulls.

Characteristic	G1	G2	Significance
Thickness (μm) of:			
Tunica adventitia	125 \pm 8.1	130 \pm 9.3	NS
Tunica musculosa	238 \pm 10.1	380 \pm 12.3	**
Interlobular septa	230 \pm 18.3	312 \pm 12.5	*
Inter-alveolar septa	42 \pm 2.1	28 \pm 2.9	*
Diameter (μm) of:			
Alveoli	93 \pm 5.1	129 \pm 4.7	**
Inter-alveolar tubules	68 \pm 4.1	76 \pm 3.8	NS
Inter-lobular ducts	93 \pm 3.8	101 \pm 4.5	NS

NS= Not significant * $P<0.05$ ** $P<0.01$

In light on the previous findings the present histometric data of the seminal vesicles may histologically indicate more developed alveolar system and functionally, more secretion activity in G2 than G1, which reflects the importance of the seminal vesicles in production of the seminal plasma.

Prostate gland:

In bulls of both groups, the prostate gland was of tubulo-alveolar type surrounding the first portion of the urethral wall. Histologically, the prostate gland was composed of connective tissue (prostatic stroma) and secretory acini and their tubules (prostatic parenchyma) and was enclosed by a thick fibro-muscular capsule. Septa (Inter-lobular trabeculae) were extending from the capsule towards the prostatic urethra dividing the prostatic mass into small lobules containing secretory acini (Figs. 6 and 7). Sudhakar, *et al.* (1985) described similar findings in Murrah bulls.

The interlobular trabeculae consisted of a dense connective tissue in G2 and a loose type in G1. Trabeculae of G1 was significantly ($P<0.05$) thicker than that in G2, as a result of compacted acini in prostatic gland of G2 bulls (Table 4 and Figs. 6 and 7).

The prostatic parenchyma was formed of acini, which were more compact in G2 than G1 (Figs. 6 and 7). Similar observations were found in adult buffalo bulls as reported by Chandramouly (1971), however, Aboul-Omran, 2005 found similar trend between fertile and infertile buffalo bulls.

It is worth noting that in G2 the acini were lined with a single layer of columnar epithelium, while in G1 the lining epithelium consisted of both columnar and cubical types. Banks (1974) observed the two types of epithelial cells in acini of bovine bulls. Average thickness of the epithelial layer of acini did not differ significantly between both groups (Table 4).

The significantly ($P<0.05$) wider acini in the pars interna of the prostate gland of G2 than G1 reflect the increased secretory activity of this gland in bulls of G2 than in G1. Concerning the tubular system, average diameter of tubules of acini showed insignificant differences between bulls in both groups (Table 4).

Cowper's glands:

It is worth noting that the histometry and histology of Cowper's glands in fertile and low-fertile buffalo bulls were nearly similar (Fig. 8). The similarity in histological findings beside insignificant differences in morphometric characteristics of Cowper's glands between bull groups indicated the limited role of Cowper's secretion in determination of semen volume of bulls (Hafez and Hafez, 2000), especially in fertile and low-fertile buffalo bulls (Aboul-Omran, 2005).

The previous results indicated the superiority of bulls in G2 in most of the morpho-histometric measures of the seminal vesicles and histometric measures of the pars interna of the prostate gland. In addition, the significant ($P<0.01$) higher level of testosterone in bulls of G2 than G1 may indicate the vital role of testosterone in development of the accessory sex glands.

Table (4): Histometry of prostate gland (Pars interna) in buffalo bulls.

Item	G1	G2	Signif.
Thickness (μm) of:			
Inter-lobular trabeculae	80.5 \pm 2.20	69.3 \pm 1.3	*
Epithelium of acini	16.5 \pm 0.21	16.2 \pm 0.23	NS
Diameter (μm) of:			
Acini	47.2 \pm 0.28	55.5 \pm 0.30	*
Tubules	29.8 \pm 0.38	29.7 \pm 0.35	NS

NS = Not significant * $P<0.05$

CONCLUSION

The present study proves a strong relationship between morpho-histometry of the seminal vesicles and histometry of the prostate gland on one side, and ejaculate semen volume of Egyptian buffalo bulls on the other side.

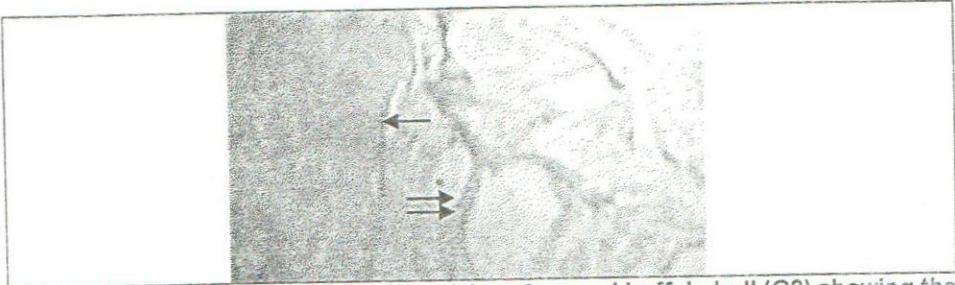


Fig. (1): Cross-section in seminal vesicles of normal buffalo bull (G2) showing the surrounding tunica adventitia (↓) and alveoli within the seminal lobules (↓↓). (H&E, x 200)



Fig. (2): Cross-section in seminal vesicles of normal buffalo bull (G2) showing compact vesicular alveoli (↓) and thin inter-alveolar stroma (↓↓). (H&E, x 150)

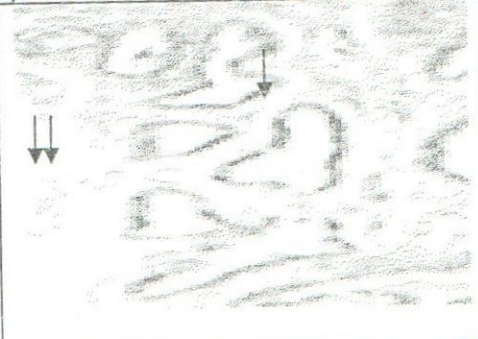


Fig (3): Cross-section in the seminal vesicles of low-semen buffalo bull (G1) showing loose vesicular alveoli (↓) and thick inter-alveolar stroma (↓↓). (H&E, x 150)

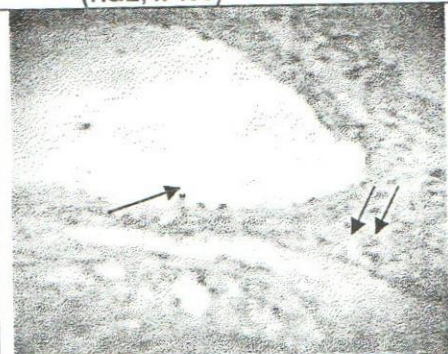


Fig. (4): Magnification of the seminal vesicles of normal buffalo bull (G2) showing large size and thick lamina epithelialis of the vesicular alveoli (↓) and more muscular fibers in inter-alveolar stroma (↓↓). (H&E, x 400)



Fig. (5): Magnification of the seminal vesicles of low-semen buffalo bull (G1) showing small size and thin lamina epithelialis of the vesicular alveoli (↓), and more fibroblasts and low muscular fibers in inter-alveolar stroma (↓↓). (H&E, x 400)

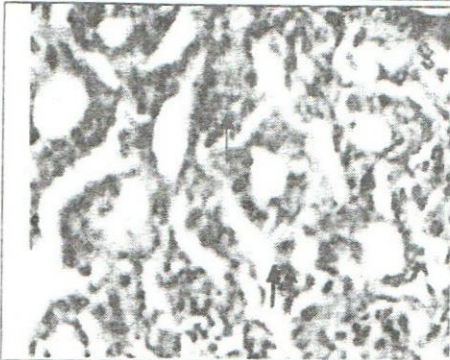


Fig. (6): Cross-section in prostate gland of normal buffalo bull (G2) showing dense connective tissue in inter-lobular trabeculae (↓) and compact wide secretory acini of prostatic parenchyma (↓↓). (H&E, x 150)

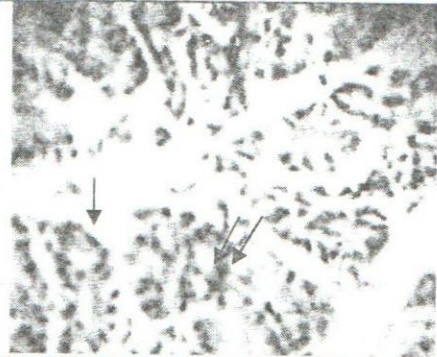
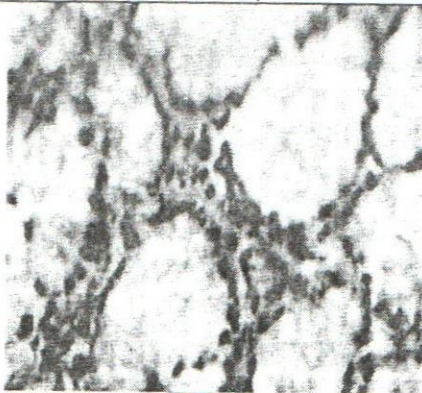
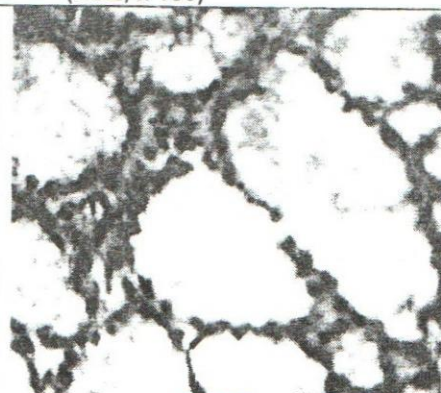


Fig. (7): Cross-section in prostate gland of low-semen buffalo bull (G1) showing loose connective tissue in inter-lobular trabeculae (↓) and less compact narrow secretory acini of prostatic parenchyma (↓↓). (H&E, x 150)



A



B

Fig. (8): Cross-section in the Cowper's glands of normal (A) and low-semen (B) buffalo bull showing quite similarity in histology of both bulls. (H&E, x 400)

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العلاقة بين القياسات المورفولوجية والهستولوجية للغدد الجنسية المساعدة وحجم السائل المنوي لطلائق الجاموس المصري

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تم دراسة الخصائص التشريحية والهستولوجية للغدد الجنسية المساعدة في أربع طلائق جاموسي منخفضة الخصوبة مع انخفاض في حجم السائل المنوي (المجموعة الأولى) مقارنة بأربع طلائق جاموسي ذات مستوى خصوبة وحجم سائل منوي عادي (المجموعة الثانية) وأخذت جميع الطلائق من قطع الجاموس بمحلة موسى التابعة لمعهد بحوث الإنتاج الحيواني. وكان وزن الجسم والعمر متشابهة تقريبا في المجموعتين وكانت جميع الحيوانات واقعة تحت ظروف غذائية ورعاية واحدة.

وتحت ظروف التجربة تأكد الانخفاض المعنوي في حجم السائل المنوي وحيوية وتركيز الحيوانات المنوية وتركيز هرمون التستوستيرون في طلائق المجموعة الأولى عن المجموعة الثانية

تم ذبح الحيوانات في نهاية التجربة وتبين الآتي:

1. كان وزن وطول وعرض الحويصلات المنوية أكبر معنويا في الطلائق العادية عن الطلائق منخفضة الخصوبة بحوالي ٦٢، ٥٤ و ٥٩%، على الترتيب، بينما لم تختلف جميع قياسات غدة البروستاتا وغدتي كوبر اختلافا معنويا بين المجموعتين.
 2. وجد ارتباط موجب ومعنوي بين حجم القذقة وكل من وزن ($r=0.94$) وطول ($r=0.58$) وعرض ($r=0.38$) الحويصلات المنوية.
 3. كان سمك الطبقة العضلية والنسيج بين الفصيصات وقطر الحويصلات الإفرازية أكبر في الحويصلات المنوية لطلائق العادية عن الطلائق منخفضة الخصوبة بحوالي ٦٠، ٣٥ و ٣٦%، على الترتيب. بينما كان النسيج بين الحويصلات الإفرازية للحويصلات المنوية أسمك معنويا في الطلائق منخفضة الخصوبة عن الطلائق العادية (٣٢ مقابل ٢٨ ميكرومتر).
 4. كان سمك النسيج البيني داخل الجزء المفرد من غدة البروستاتا أكبر معنويا في الطلائق منخفضة الخصوبة عن الطلائق العادية بحوالي ١٦%، بينما أنخفض قطر الحويصلات المفردة معنويا في المجموعة الأولى عن الثانية (٤٧,٢ مقابل ٥٥,٥ ميكرومتر).
- أثبتت هذه الدراسة وجود علاقة قوية بين القياسات المورفولوجية والهستولوجية للحويصلات المنوية والقياسات الهستولوجية لغدة البروستاتا مع حجم السائل المنوي لطلائق الجاموس المصري، بينما لم يتأثر حجم القذقة بالقياسات المورفولوجية والهستولوجية لغدد كوبر.

