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Evaluation of testicular outcomes following laparoscopic assisted orchidopexy in dogs

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ABSTRACT

Cryptorchidism is a common congenital abnormality encountered in dogs. The commonly used treatment for this condition is orchiectomy or orchidectomy. Surgical placement and fixation of the cryptorchid testicle into the scrotum, referred to as orchiopexy, is used in humans. The treatment of cryptorchidism by orchiopexy in dogs is a controversial topic due to the hereditary nature of cryptorchidism in dogs. The present study was focusing for the first time on long-term monitoring of the testicular soundness and function following laparoscopic orchidopexy in young dogs. Seventeen (n.= 17) German shepherd young dogs (180±14 days) were included in the study. All cases enrolled in this study underwent laparoscopic testicular descent and orchidopexy. Ten days postoperatively, the dogs were evaluated by Doppler ultrasound to assess the testicular blood flow. At age of 18-20 months, semen parameters were evaluated in all treated dogs. Clinically, the abdominal wounds were healed 3-4 days after surgery with complete absence of all signs of infection, pain or discomfort. Testicular volume (TV; cm3) and thickness of the mediastinum line (MT; mm) were within normal values (8.65±0.25 cm3 and 1.11±0.85 mm), with normal testicular blood flow. The mean values of semen parameters were (82.68% for individual motility and 90.35% for the percentage of live sperm). In conclusion, laparoscopic descent and orchidopexy can be utilized with keeping the normal testicular functions when performed at young, aged dogs (180±14 days).

Introduction

Unilateral or bilateral absence of scrotal testes in dogs may lead to a clinical issue at certain age. Cryptorchidism is a hereditary condition and is one of the most common congenital defects in small animal practice (Yates *et al*., 2003). Scrotal testicular descend occurs in behalf of fibrotization and shortening of the gubernaculum ligament under fetal androgenic control during the 54th day of pregnancy (Yates *et al*., 2003). Testicular passage through inguinal canal takes place within the 5th day following birth and the completed testicular descend in the scrotum could be finalized and observed in puppies at 6-8 weeks following birth (Kawakami *et al*., 1993). Up to the age of six months, partial sealing of the inguinal canal occurs that could delay further passage of the abdominal testes (Johnston *et al*., 2001).

The definitive reason of cryptorchidism in dogs has not been fully illuminated but it seems to be multifactorial (Romagnoli, 1991). Abdominal testes are predisposing to certain pathological states like testicular torsion, in which spermatic cord rotates around its longitudinal axis making acute pain (Quartuccio *et al*., 2012) and testicular tumors, which develop 13.6 times greater than scrotal testes (Khan *et al*., 2018). Ultrasonographic scanning could reveal the retained testis in the abdominal or inguinal or prescrotal regions (Matoon and Nyland, 2015). In few cases, ultrasonographic scanning fails to accurate determination of the testes position, so laparoscopy considered a gold standard investigation for identifying the testicular location and then making the laparoscopic intervention for orchidopexy (Pettersson *et al*., 2007).

Laparoscopically guided intervention for cryptorchid testes in dogs provides many merits as it is less invasive approach, lower herniation risk, visualizes abdominal and inguinal organs and shorter operation time that reflects on shorter exposure to anesthesia (Miller *et al*., 2004). The testicular functioning outcome depends firstly on the vasculature system that conducts hormones and nutrients, Color and pulsed Doppler ultrasonography is widely used in dogs for testing testicular haemodynamics and blood flow (Abdelnaby *et al*., 2021a; Salama *et al*., 2022), that will be reflected on the processes of steroid oogenesis and spermatogenesis.

In humans, cryptorchidism is a common finding affecting newly born males and has an impact on spermatogenesis and incidence of testicular malignancy, so orchidopexy is applied to ameliorate these effects (Pettersson *et al*., 2007).

In dogs, many successful trials were reported for laparoscopic cryptorchidectomy (Lew *et al*., 2005), while the current work is focusing for the first time on the laparoscopic orchidopexy instead of cryptorchidectomy with giving the advice for the dog owners to prevent the future breeding of these treated dogs as they will be genetically predisposed to this condition. From this point of view, the present study aimed at monitoring the testicular function following laparoscopic descent and orchidopexy through the assessment of testicular blood flow following orchidopexy and later by assessment of semen parameters.

Materials and methods

Animals

This study was approved by the ethical committee at Faculty of Veterinary Medicine, Cairo University, Egypt. All dogs were treated in accordance with the guidelines established by the international and institutional Animal Care and Use Committees (Vet Cu 03162023678). All patients were examined and treated surgically at the Theriogenology Department, Faculty of Veterinary Medicine, Cairo University from January 2019 to January 2023. Informed consent was obtained from the owners of all enrolled dogs before performing the diagnostic and treatment procedures for the possibility of surgery difficulties or intraoperative complications.

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Seventeen German shepherd dogs (180±14 days old) were included in the present study; twelve dogs were admitted with unilateral cryptorchidism and the rest five were bilaterally cryptorchid.

Study design

Males with either unilateral or bilateral cryptorchidism were prepared for laparoscopic testicular descent and orchidopexy after their owners' approval. Ten days following laparoscopic orchidopexy, dogs were exposed to Doppler examination to assess the testicular blood flow. At age of 18-20 months, the treated dogs were subjected to semen collection and evaluation of semen parameters.

Assisted laparoscopic testicular descent and orchidopexy

Anesthesia

Dogs were routinely pre-medicated with atropine sulphate (Atropine sulphate®; ADWIA, Egypt) and xylazine hydrochloride 2% (Xylaject®; ADWIA, Egypt) in a dose of 0.04 mg/kg b.w. s/c. and 1 mg/kg b.w. i.v., respectively. General anesthesia was achieved with ketamine hydrochloride 5% (Keiran®, EIMC pharmaceuticals Co., Egypt) in a dose of 15 mg/kg b.w. i.v. (Farghali *et al.*, 2017).

Surgical Technique

Complete aseptic precautions and pre-operative fasting were endorsed for all dogs enrolled in this study. For better visualization, a urinary bladder catheter was applied, and the urine was evacuated before surgery. The dogs were positioned in a dorsal recumbency.

The following equipment were used in the surgeries; a source of cold light (xenon light source, Storz, Germany), with a video camera and CO_2 insufflator and a 10 mm laparoscopic telescope with 0° visual angle.

An induced- pneumoperitoneum was applied to obtain the operative space using carbon dioxide at a pressure of 12 mmHg. The laparoscopic ports were created at the points where the edges of an isosceles triangle meet. The top point of the triangle was identified by an 11 mm optical trocar (the first port). It was placed in a white line, 2 cm posteriorly from the umbilicus. Two additional ports (2nd port; 11 mm trocar and 3rd port; 5mm trocar) were placed either on the right or left side; about 8 cm away from the first port in the posterior direction near the white line (Lew *et al.*, 2005).

The cryptorchid testicles were located intra-abdominally and were freed by dissecting the gubernaculum using a pair of monopolar scissors. Dissection was done cranially until sufficient length of the spermatic cord was obtained to perform orchidopexy without tensing it. By using grasping forceps, they were grasped till inguinal ring. A 2 cm scrotal incision was made in the scrotum corresponding to the undescended testis and a subdartos pouch developed. A 5 mm trocar was passed from the scrotal incision into the abdomen. The testis was grasped with grasping forceps. The testis was brought out into and fixed in the scrotum. The scrotum wound was then closed using # 4 - 0 polyglactin (Coated Vicryl®; Ethicon, USA), as shown in Figures 1, 2, 3 and 4.

Post-operative pain and infection were controlled using systemic course of antibiotic and anti-inflammatory/pain killer. The course was conducted for 7 successive days with Cefotaxime sodium at a dose of 10 mg/kg and meloxicam in a dose rate of (0.05 mg/kg) during the first three days after surgery.

Ultrasonographic (B- mode and Doppler) evaluation

Both testes were examined ultrasonographically (applied anatomy procedure) on day 10 post-operatively. The technique of examination was performed using ultrasound device (EXAGO, France), with a linear probe and 7.5 MHz frequency. Dog was positioned in a dorsal recumbency, and adequate amount of acoustic gel was applied to minimize air artifact in the scanning, at first, the probe was placed on the testicular tissue at the lateral surface in order to measure length (L), height (H) via longitudinal plane, and width (W) via a transverse plane (Figure 5 A and B) with marked visualization of mediastinum line (as a reference line to make perfect view) at the longitudinal view. The testicular volume (TV; cm3) was now easy calculated by the following equation using in the ellipsoid organ as follows: TV= L (cm) × H (cm) ×W (cm) × 0.5236 (Salama *et al.*, 2022). The testicular parenchyma and echotexture were measured by frozen testicular tissue B – mode image via testicular echogenicity (TE; numerical pixel value [NPVs]), and pixel heterogeneity (PH; standard deviation of numerical pixel value [sd NPVs]).

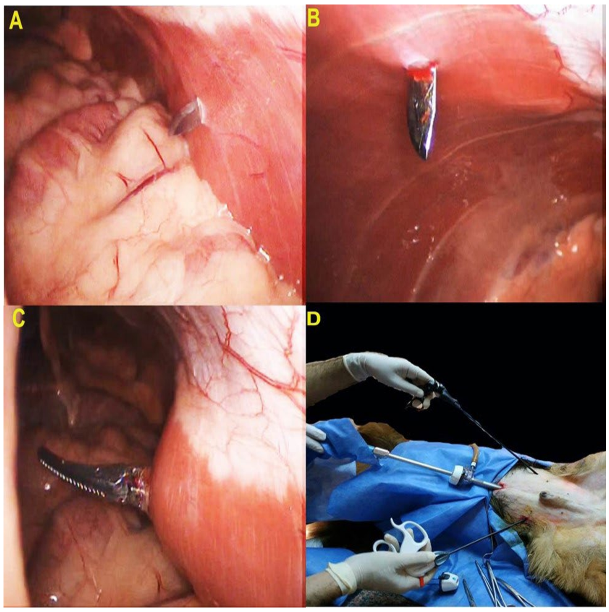

Figure 1. A photograph showing piercing the abdominal cavity 2 cm posteriorly from the umbilicus then from left side and right side through the abdominal muscles' layers (A, B and C). Introducing the laparoscopic ports (D).

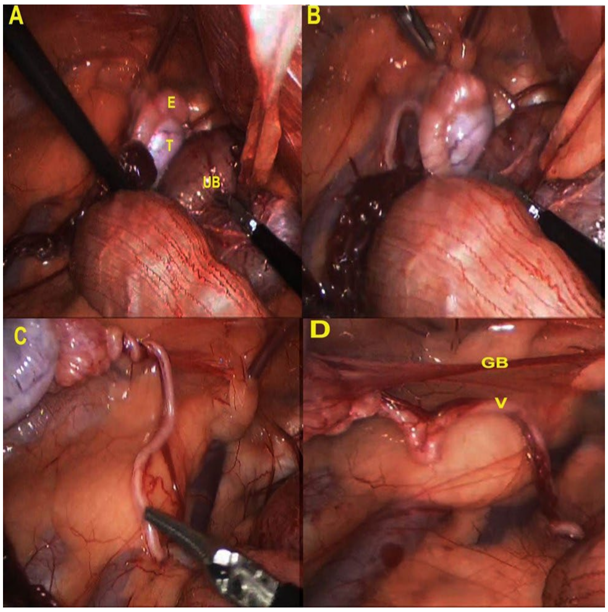

Figure 2. A photograph showing a twisted testis in the abdominal cavity (A, B, C). Untwisting was completely performed (D). E: epididymis, T: testis, UB: urinary bladder, GB: gubernaculum ligament, V: vas deferens.

The testicular artery vascular perfusion was estimated via activated color mode to see the colors (red and blue) that determine the direction of flow either toward or away the transducer (Abdelnaby and Emam, 2022).

The measurement of the artery was at two definite regions , the first one was at the level of pampiniform plexus (Figure 5 C) to estimate the distal branch of supra testicular artery (Distal STA; Figure 5 D) at the cranial pole of the testis (Farghali *et al.*, 2017), by placing the probe at the neck of the scrotum to show the tortuous region (looping), while the second region, the probe moved on the testes distally at the dorsal view to estimate the marginal artery (Marginal TA; Figure 5 E) that appear linear straight artery. The spectral mode was activated by a window with a gate size 1mm to enter inside the vessel lumen ,and three successive waves characterized by a peak point of velocity (PV; cm/sec), end point of velocity (EV; cm/sec), time average to obtain maximum velocity (TAV ;cm/sec), and rate of blood flow (BFR; bpm) to calculate both Doppler indices as resistance index (RI) and pulsatility index (PI) with a PRF = 4000k (Daghash *et al.*, 2022).

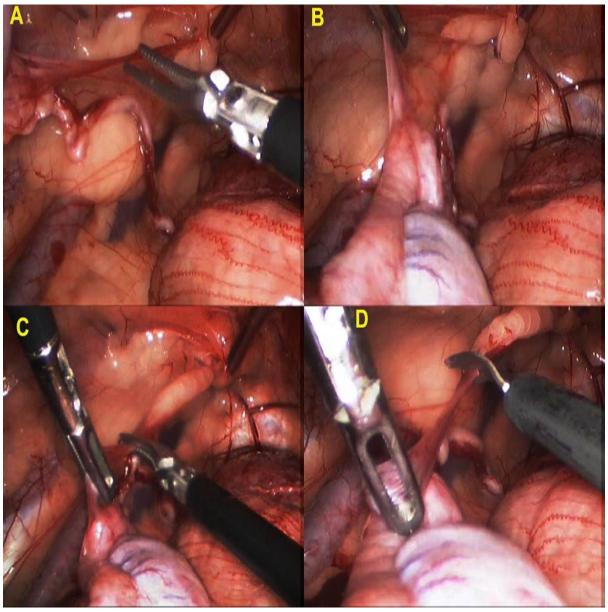

Figure 3. (A, B, C and D): A photograph showing the steps of dissecting the grasped gubernaculum ligament.

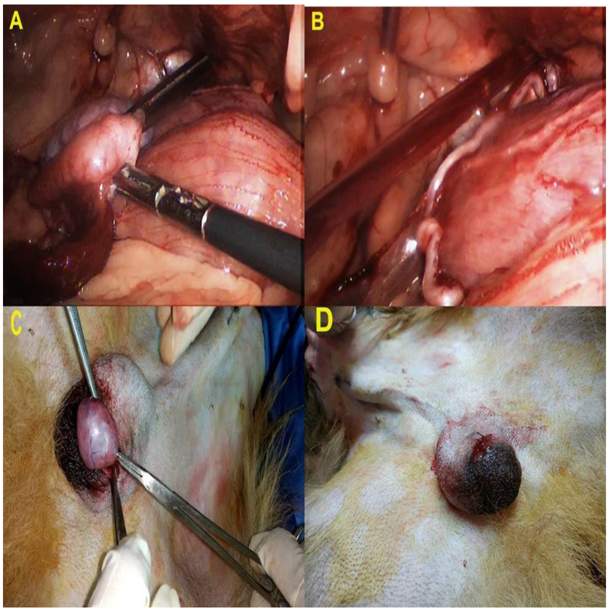

Figure 4. (A and B) A photograph showing grasping the testicle toward the inguinal ring. C) The testis was brought out into and fixed (Orchidopexy) in the scrotum, D) suturing the scrotal wound.

Semen collection and evaluation

Semen samples were collected by digital manipulation (Freshman, 2002), forward motility of sperm was immediately recorded by adding a drop of semen sample with a drop of prewarmed 37ºC tris buffer on a clean slide using phase-contrast microscope supplemented with hot stage at 400 X magnification (Freshman, 2002).

Percentages of live spermatozoa were assessed using eosin-nigrosine staining through counting 200 sperm under 1000 X magnification; sperm cell concentration was calculated using haemocytometer (Albrizio *et al.*, 213).

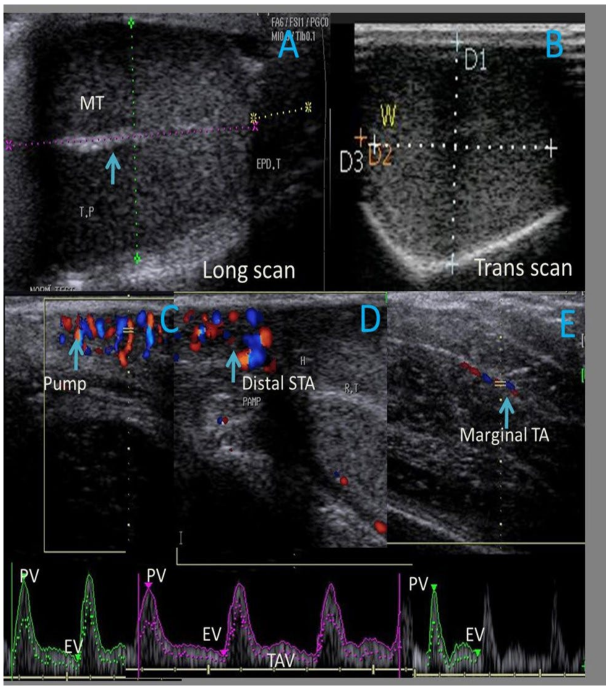

Figure 5. B - mode and colored ultrasonograms of testes and testicular artery. A: a longitudinal ultrasonographic scan showing; length (L) and height (H) in with an appearance of mediastinum testis and its thickness (MT) by B-mode , B: a transverse scan of testis to measure testicular width (W) in order to calculate the testicular volume, C: an appearance of pampiniform plexus (pump) with a blue arrow .D: appearance of the distal branch of supra-testicular artery (STA) with a blue arrow and its spectral graph showed both Doppler velocities as peak and end point of velocities (PV and EV). E: appearance of another branch for testicular artery called marginal testicular artery with a blue arrow (TA).

Statistical analysis

Data was checked first for normality by Shapiro - Wilk test in the descriptive statistics. Data are presented as mean±standard deviation (SD). All normal parameters in testicular morphometric and hemodynamics were checked by SPSS (2007), with a Duncan multiple test for the significant difference at probability < 0.05. Semen quality parameters were analyzed by one way ANOVA followed by Tukey's multiple comparison test, data were recorded as mean±SEM.

Results

Clinical findings

All laparoscopic surgeries were performed without any recorded complications; just appeared by surface anatomy and diagnosed by palpation of scrotum.

The elapsed time for laparoscopic orchidopexy was nearly 23.75±0.59 minutes (range, 21 - 27 minutes) in unilateral cryptorchid males and nearly 37.1±0.59 minutes (range, 33-40 minutes) for bilaterally cryptorchid males. The next day following laparoscopic surgeries, dogs felt active with good appetites, abdominal wounds left by ports of laparoscope were healed within 3-4 days with no characteristic swelling or redness.

B-Mode testicular characterization

The duration for clinical, and ultrasound examination (B - and Doppler) was 20 minutes with a range (10-30 minutes).Testicular volume (TV; cm^3) and thickness of the mediastinum line (MT; mm) were lie within normal values (8.65±0.25 cm^3 and 1.11±0.85mm), receptively, with their ranges (7-10 cm^3 for TV and 1.05-1.15 mm for MT). In addition, testicular echogenicity (TE), and pixel heterogeneity (PH) were also lie within normal values (88.5±5.32 and 15.58±0.78), all data were obtained as mean and standard deviation. Both right and left testes were recorded as homogenous in appearance and the mediastinum testis was identified totally 100 % at each examination as white hyper echogenic line (Figure 5A).

Color Doppler testicular artery characterization at two different regions

When pampiniform plexus is visualized via ultrasound scanner by activating color mode (Figure 5C), we can easily identify the distal supra testicular artery (Distal SPA; Figure 5D) in all dogs at each assessment, as this branch of the testicular artery was tortuous in pattern, while the marginal artery was identified as a more linear pathway with a straight pattern (Figure 5E). Both branches showed a monophasic pattern of blood flow cardiac cycle in the spectral graph (Figure 5D, and Figure 5E), no significant differences were observed between both testes ($p > 0.05$). Both end point of velocity and time average velocity (EV and TAV; cm/sec) did not differ in both branches (Distal STA and Marginal TA). These values resulted in differences in peak point of velocity (PV; cm/sec) and blood flow rate (BFR; bpm) as those two parameters were within normal values and significantly declined ($p < 0.05$) in marginal branch (12.09±0.21 and 59.32±0.71) compared to their value in Distal STA (14.55±0.66 and 66.25±0.33; Table 1), while both Doppler indices as resistance index and pulsatility index (RI and PI) were significantly elevated ($p < 0.05$) in marginal TA than in distal STA (Table 1).

Table 1. Testicular arteries hemodynamics evaluated by Doppler ultrasound (color and spectral modes).

Doppler parameter variables	Distal STA	Marginal TA
PV (cm / sec)	14.55±0.66[a]	12.09±0.21[b]
EV (cm / sec)	5.66±1.02	5.36±0.25
TAV (cm / sec)	2.25±0.82	2.19±0.05
BFR (bpm)	66.25±0.33[a]	59.32±0.71[b]
RI	0.52±0.02[b]	0.88±0.36[a]
PI	1.05±0.01[b]	1.22±0.01[a]

PV = peak point of velocity, EV = end point of velocity, TAV = time average to obtain maximum velocity, BFR = blood flow rate, bpm = beat per minute, STA = supra testicular artery, TA = testicular artery, RI = resistance index, and PI = pulsatility index. Different superscript letters (a and b) represent significant differences in the variable between two regions of the testicular artery ($p < 0.05$).

Evaluation of semen characteristics for all treated dogs

At the age of 18-20 months, all males (n = 17) were subjected to semen collection and analysis, the mean values of semen parameters was 82.68% for individual motility and 90.35% for the percentage of live sperm.

The mean value of sperm cell concentration was 260×10^6/ml, as shown in Table 2.

Table 2. Semen parameters for all treated dogs (n.= 17).

Semen parameter	Mean±SEM
Individual motility (%)	82.68±3.26
Live (%)	90.35±6.38
Sperm cell concentration (10^6 / ml)	260±12.56

Data are presented as mean±SEM.

Discussion

Cryptorchidism is one of the most reproductive disorders affecting male dogs; many previous trials had been recorded to resolve this problem by laparoscopic orchidectomy in dogs (Lew *et al.*, 2005), while in humans, several trials were done for laparoscopic orchidopexy (Pettersson *et al.*, 2007).

So, it was the first trial in dogs to resolve this problem by laparoscopic orchidopexy and monitoring the testicular outcome following orchidopexy.

Laparoscopically assisted orchidopexy provided many advantages; monitoring abdominal and inguinal organs, safely determination of testes position, lesser operation time and lower herniation risk (Miller *et al.*, 2004). The clinical examination of dogs after laparoscopic orchidopexy had been showed good soundness with normal pulse and temperature a day following operation, 3-4 days following the operation, healing of the abdominal wounds was noticed with no post-operative reaction, these findings were parallel to the clinical findings reported by Lew *et al.* (2005) that made laparoscopic orchidectomy.

The findings in this current study provide adequate and further insight in to testicular hemodynamics pattern after laparoscopic treatment of cryptorchidism in dogs; this study reported the testicular morphometric analysis by B - mode in form of TE, PH and MT. Similarly, many studies reported the elevation in MT in post-pubertal animals which is closely related to TE and PH that directly linked with animal age and maturity with normal appearance of testicular morphometry (Abdelkhalek *et al.*, 2022). We observed only two regions for the testicular artery as distal SPA and marginal one, as the proximal branch doesn't observed as there was a difficulty to ensure consistency of position between dogs (Souza *et al.*, 2014), the Doppler parameters including velocities and blood flow rate were found to be reproducible and did not change all over the study after intervention, as these results appear to offer enhanced reliability of Doppler imaging compared with other studies (Gumbsch *et al.*, 2002). All dogs had monophasic Doppler testicular artery blood flow pattern in the spectral wave obtained via pulsed wave mode with a distinct systolic peak (PV), continuous diastolic flow (EV), and low vascular resistance (RI) with pulsatility (PI). These outcomes are comparable to those that have already been reported for the marginal and supra-testicular arteries (Carrillo *et al.*, 2011; Günzel *et al.*, 2001). The blood flow profiles matched those previously seen in human and canine testes (Middleton *et al.*, 1989; Salama *et al.*, 2022), and then compared which flow that need a continual perfusion (Carvalho *et al.*, 2008).

However, the TAV and EV in the current study were similar in magnitude and in two branches of the testicular artery similar to those reported before (Carrillo *et al.*, 2011), despite the fact that absolute measurements of arterial blood velocity are clearly difficult to compare between studies due to different equipment, patients, and technical factors. The marked inverse relationship that observed between PV and Doppler indices were studied in many reports (Fadl *et al.*, 2022; Abdelnaby *et al.*, 2021b), as RI is a computed value and is only a genuine depiction of downstream vascular resistance when resistance is the only variable, contrary to what one may intuitively anticipate, which is that it would decrease during sexual maturity associated with vasodilation and increased blood flow (Souza *et al.*, 2014).

At maturity age, the collected semen was evaluated and revealed values within the normal range concerning with motility, viability and sperm cell concentration, these values were similar to those previously recorded in many reports (Salama *et al.*, 2022; Freshman, 2002) they recorded that the mean values of sperm concentration were ($177\text{-}230\times10^6$/ml) and the motility percentage was (70-80%) for the control group.

Despite these benefits of laparoscopic orchidopexy but the dog owner should be advised to prevent future breeding of treated males, and also advised to make laparoscopic orchidectomy in cases of either old aged cryptorchid males or in cases of neoplastic retained testes.

Conclusion

Despite the laparoscopic orchidectomy of the cryptorchid testes had been well recognized in dogs, laparoscopic descent and orchidopexy can be utilized with keeping the normal testicular functions when performed at young dogs. Laparoscopic orchidopexy also can protect the cryptorchid dogs from the expected consequences like testicular malignancy or intra-abdominal testicular torsion, but with giving the advice for the dog owners to prevent their future breeding.

Conflict of interest

The authors declare that there is no conflict of interest

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