



Comparative evaluation of pinhole and section ligation release castration techniques in male dogs

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Received: 22 August 2022; Accepted: 14 September 2023

ABSTRACT

The present study was carried out to compare two minimally invasive surgical *in situ* castration techniques in dogs. Male dogs (n-12) brought to the department for elective castration were randomly divided into two groups comprising six in each group. In Group I, pinhole (PH) castration, and section ligation release (SLR) castration in group II were performed. Physiological parameters, testicular dimensions, haemato-biochemical analysis, ultrasonographic examination and pain scores were recorded on 0th, 3rd, 14th and 28th day. Testicular tissue biopsy sample for histopathological examination were taken on 0th and 28th day. Physiological parameters, testicular breadth and height revealed non-significant variation, while significant difference was observed in testicular circumference and volume in both the groups. Non-significant difference was noted in haemoglobin, packed cell volume, total leucocyte count and differential leucocyte count within or in between the groups. Cortisol and MDA were significantly higher on 3rd day, whereas GSH was significantly lower on 3rd day in comparison to other time intervals in both the groups. Pain scores were significantly higher on 3rd day in both the groups and were also significantly more in group II. Testosterone levels were markedly reduced in both the techniques, but values were significantly lesser in group II as compared to group I. Ultrasonographic and histopathological examination revealed marked testicular degeneration in both the techniques. It was concluded that pinhole castration technique is minimally invasive, less time consuming, economical and has less post-operative complications in comparison to section ligation and release technique.

Keywords: Castration, Dog, Pinhole, Section ligation release

Veterinary epidemiologists and parasitologists are primarily concerned with the regulation of dog populations, as they are responsible for the spread of more than 60 zoonotic diseases (Kutzler and Wood 2006, Rinaldi *et al.* 2006). Conventional surgical sterilization is considered stressful, expensive and has more post-operative complications (Okwee-Acai *et al.* 2012, Baba *et al.* 2013). Other castration procedures such as scrotal ablation (Misk and Seleim 1987) and chemical castration have also been described. In chemical castration, irritant chemicals such as calcium chloride, zinc gluconate, lactic acid, silver nitrate, ethanol, phenol, formalin, etc. are injected into the testicles, resulting in permanent infertility (Sobhy *et al.* 2016). However, complications such as testicular swelling, hydrocele, scrotal ulcer, fistula formation, scrotal prolapse and lameness can result from this castration method (Al-Asadi and Al-Kadi 2012). Hormonal or immunocastration has recently developed as a technique that triggers less physiological stress, but this technique is not reliable as it

does not provide the option of long-term castration (Cohen *et al.* 1990). It also has technical difficulties, a requirement for repeated shots, and owner concerns. In addition, keyhole surgery (laparoscopic vasectomy) results in early healing (Mahalingam *et al.* 2009) but requires expensive equipment, expertise and is time consuming, so it cannot revolutionize the entire Animal Birth Control program. Thus, there is a need to develop an alternative animal sterilization technique that is simple, rapid, feasible and less stressful to the animal (Ponvijay 2007). Among other minimally invasive techniques, alternative *in situ* methods called pinhole and section ligation release have yielded more favourable results in ruminants (Ponvijay 2007, Okwee-Acai *et al.* 2008, Fazili *et al.* 2009) and horses (Saifzadeh *et al.* 2008).

MATERIALS AND METHODS

The present study was conducted on 12 male dogs presented for elective castration irrespective of age, breed and body weight, which were randomly divided into two groups comprising six in each group. In both the groups, animals were restrained in dorsal recumbancy and scrotal area was aseptically prepared prior to surgery. Animals were

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premedicated with atropine sulphate @0.04 mg/kg b.wt I/M and then anaesthesia was induced using the combination of Inj. xylazine hydrochloride @1 mg/kg b.wt and ketamine hydrochloride @10 mg/kg b.wt intramuscularly. Surgical anaesthesia was maintained using combination of xylazine and ketamine intravenously in 1:1 ratio in both the groups.

Group-I: Pinhole castration technique: In this technique, spermatic cord was grasped with the help of fingers and thumb. A 16-gauge hypodermic needle with suture material was passed below the spermatic cord (Fig. 1A). Now leaving the suture material at place, needle was removed (Fig. 1B). Then again needle was reintroduced through previously made skin holes but this time above the released spermatic cord (Fig. 1C and D). Hypodermic needle was removed while keeping the suture on spermatic cord. This led to the formation of loop around the cord (Fig. 1E). Both the free ends of sutures were tied (Fig. 1F) and transected close to the scrotum. Thereafter, the needle holes were only visible immediately after submerging the knot inside the scrotal skin. Similar procedure was followed for other spermatic cord.

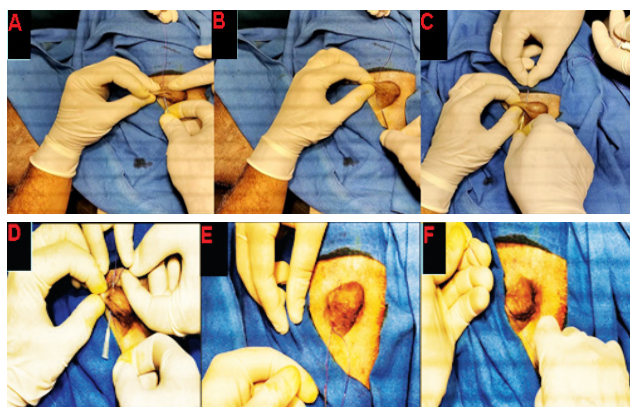


Fig. 1. Pinhole castration technique.

Group-II: Section ligation release castration technique: In this technique, spermatic cord was grasped within the fingers and thumb. A longitudinal skin incision was given directly above the cord (Fig. 2A and B). After incising the skin, fascia was removed, and spermatic cord was exteriorized using small mosquito artery forceps by placing

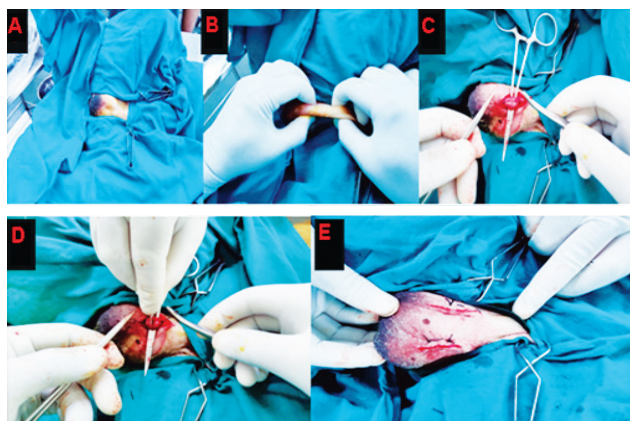


Fig. 2. Section ligation release castration technique.

it below the cord (Fig. 2C). Then two simple interrupted sutures were placed over the spermatic cord using vicryl no.1, one cranial and caudal to artery forcep, then suture ends were held using artery forceps. Then spermatic cord was incised between the two sutures and was checked for any bleeding (Fig. 2D). Artery forcep was then released and subcutaneous tissue was sutured using vicryl no.1. Skin was sutured using horizontal mattress suture pattern with non-absorbable silk no.1 (Fig. 2E). Similar procedure was performed on the other spermatic cord.

Following parameters were evaluated in both the techniques.

History: Detailed history of the animal, i.e. age, breed, body weight and any pre-existing illness (if any) were taken on the day of presentation.

Physical and clinical examination: Physiological parameters, viz. rectal temperature ($^{\circ}\text{F}$), heart rate (beats per min), respiratory rate (breaths per min), scrotal and testicular dimensions were recorded on 0th, 3rd, 14th and 28th days postoperatively.

Haematological analysis: Haematological parameters, i.e. haemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC), packed cell volume (PCV) and differential leucocyte count (DLC) were estimated using automated haematological analyser.

Pain scores: Pain score assessments were performed using Glasgow composite measure pain scale on 0th, 3rd, 14th and 28th day postoperatively.

Oxidative stress: Malondialdehyde (MDA) concentration was estimated by standard procedure suggested by Placer *et al.* (1966). The concentration of glutathione (GSH) was estimated by method suggested by Beutlers *et al.* (1963).

Hormonal analysis: Plasma cortisol and testosterone were estimated using commercially available ELISA kits.

Ultrasonographic examination: Ultrasound examination of testes was performed on 0th, 3rd, 14th and 28th days. Ultrasonographic images and pixel values were used for the evaluation of inflammation and probable lesions noticed during the present study.

Histopathological examination: Testicular biopsy samples were obtained using automatic biopsy gun. Samples were taken on 0th and 28th postoperative days.

Surgical time: Time taken to perform both the surgery by different techniques was noted and compared with each other.

Statistical analysis: The data obtained was analyzed using two-way analysis of variance (ANOVA) followed by Duncan test using SPSS 16.0 version. The level of statistical significance for all comparisons was established at $p < 0.05$. The values obtained were compared between and within the groups.

RESULTS AND DISCUSSION

History: The Mean $\pm$ SE values of age and body weight were 3.20 ± 1.06 , 1.87 ± 1.10 years and 17.33 ± 1.86 , 18.33 ± 2.70 kg in group I and II, respectively. Out of 12 dogs, the breed of animals in group I were mongrel ($n=6$),

while animals in group II were bully (n=1) and mongrel (n=5).

Physical and clinical examination: There was non-significant difference within and in between the groups in mean values of rectal temperature, heart rate and respiration rate. The results of the current study are in accordance with Khade (2006) for rectal temperature and respiratory rate. Mahalingam *et al.* (2009) also reported non-significant change in heart rate and respiration rate. Rectal temperature, heart rate and respiration rate did not change significantly after surgery, so these variables cannot be considered useful in detecting postoperative pain. The mean scrotal circumference on 3rd day was found to be significantly ($p<0.05$) higher in comparison to 0th day, decreases on 14th day non-significantly ($p>0.05$) and then significantly lower on 28th day in group I, whereas in group II, mean circumferential value on 3rd day was significantly ($p<0.05$) higher as compared to 28th day. The mean scrotal circumference value increased on 3rd day and then decreased on 14th and 28th day non-significantly. Also, there was no significant ($p>0.05$) difference observed in mean scrotal circumference, mean testicular volume and mean testicular length among the groups as depicted by Table 1. In both the groups, there was non-significant ($p>0.05$) increase in mean testicular volume (cm^3) on 3rd day and then declined non-significantly. There was significant decrease on 28th day in comparison to 3rd day in both the groups. The mean testicular length was significantly ($p<0.05$) and non-significantly ($p>0.05$) higher on 3rd day in comparison to 0th and 28th day was observed in both the groups. A non-significant ($p>0.05$) increase was noticed in mean testicular breadth and height in both the groups on 3rd and 14th postoperative day in comparison to 0th day which may be due to inflammation. These findings are in accordance with Baba *et al.* (2013) who found highest values of testicular

dimensions on 3rd day and minimum values on 28th day after pinhole castration in dogs. Similarly, Saifzadeh *et al.* (2008) observed maximum scrotal dimensions on 3rd day in equines undergone section ligation and release castration. Okwee-Acai *et al.* (2008) and Mir *et al.* (2018) also observed similar results in caprine and ponies, respectively. Atrophy of the testicular tissue was indicated by the highly significant decrease in the SC, TL and TW of the ligated testes. Atrophy is a result of spermatic cord ligation, rendering them inoperable. Tissue necrosis resulted due to total ischemia brought on by ligation (Bergh *et al.* 2001). Scrotal circumference and testicular size were closely connected to daily sperm output, sperm reserve, serving capacity, and the age at which the progeny reaches puberty. According to research on rats with spermatic cord ligation, acute testicular ischemia lasting just 5 h followed by reperfusion is enough to permanently harm spermatogenesis, primarily by apoptosis (Mir *et al.* 2018).

Haematological estimations: Mean values of Hb, PCV and TEC in both the groups as well as within the groups manifested no significant difference at different time intervals and in between both the groups. All the values were within the normal limits. A non-significant increase ($p>0.05$) in TLC and neutrophil counts whereas non-significant decrease ($p>0.05$) in lymphocytes was noticed in both the groups on 3rd post castration in comparison to 0th, 14th and 28th day. Khade (2006) also observed non-significant change in Hb, TEC and PCV along with non-significant increase in total leucocyte and neutrophils, while non-significant decrease in lymphocytes on 4th day post-castration after castration by either open castration or spermatic cord ligation in dogs.

Pain scores: Pain score was found to be significantly ($p<0.05$) higher on 3rd day as compared to 0th day and then decreased significantly ($p<0.05$) on 14th and 28th day

Table 1. Various parameters recorded at different time intervals in both the groups during castration in male dogs (Mean $\pm$ SE)

Parameter	Groups	0 th day	3 rd day	14 th day	28 th day
Scrotal circumference (cm)	Group I	12.45 $\pm$ 1.02 ^{AB}	16.13 $\pm$ 1.60 ^C	14.55 $\pm$ 1.28 ^{BC}	10.58 $\pm$ 0.88 ^A
	Group II	12.98 $\pm$ 1.16 ^{AB}	16.72 $\pm$ 1.39 ^B	14.73 $\pm$ 1.30 ^{AB}	10.73 $\pm$ 0.98 ^A
Testicular volume (cm^3)	Group I	10.18 $\pm$ 2.45 ^{AB}	21.82 $\pm$ 5.71 ^B	18.52 $\pm$ 4.03 ^{AB}	8.48 $\pm$ 2.86 ^A
	Group II	12.63 $\pm$ 3.46 ^{AB}	25.05 $\pm$ 5.78 ^B	16.90 $\pm$ 5.81 ^{AB}	9.39 $\pm$ 3.47 ^A
Pain scores	Group I	2.17 $\pm$ 0.31 ^{Bb}	5.50 $\pm$ 0.34 ^{Ca}	2.50 $\pm$ 0.43 ^B	0.17 $\pm$ 0.17 ^A
	Group II	2.33 $\pm$ 0.61 ^{Aa}	6.17 $\pm$ 1.35 ^{Bb}	2.50 $\pm$ 0.81 ^A	0.83 $\pm$ 0.48 ^A
MDA (nmol/gm)	Group I	1.35 $\pm$ 0.38 ^A	4.62 $\pm$ 0.65 ^B	2.02 $\pm$ 0.41 ^A	1.57 $\pm$ 0.38 ^A
	Group II	1.42 $\pm$ 0.31 ^A	5.44 $\pm$ 0.69 ^B	2.95 $\pm$ 0.67 ^A	1.56 $\pm$ 0.34 ^A
GSH (mg/dl)	Group I	31.28 $\pm$ 1.37 ^B	23.14 $\pm$ 1.61 ^A	30.18 $\pm$ 1.56 ^B	32.68 $\pm$ 1.85 ^B
	Group II	30.77 $\pm$ 1.30 ^B	18.03 $\pm$ 1.23 ^A	27.98 $\pm$ 1.84 ^B	31.55 $\pm$ 1.71 ^B
Cortisol (nmol/L)	Group I	58.18 $\pm$ 11.23 ^A	84.34 $\pm$ 10.25 ^B	55.07 $\pm$ 11.87 ^A	53.36 $\pm$ 8.38 ^A
	Group II	55.08 $\pm$ 12.93 ^A	88.52 $\pm$ 12.46 ^B	51.74 $\pm$ 10.20 ^A	49.13 $\pm$ 9.25 ^A
Testosterone (ng/ml)	Group I	3.72 $\pm$ 1.27 ^B	0.30 $\pm$ 0.13 ^{Ab}	0.13 $\pm$ 0.06 ^{Ab}	0.05 $\pm$ 0.03 ^A
	Group II	4.63 $\pm$ 1.21 ^B	0.03 $\pm$ 0.01 ^{Aa}	0.02 $\pm$ 0.00 ^{Aa}	0.01 $\pm$ 0.00 ^A
Pixel values	Group I	189.08 $\pm$ 9.47 ^C	159.33 $\pm$ 10.64 ^B	123.42 $\pm$ 6.74 ^A	96.92 $\pm$ 1.83 ^A
	Group II	195.33 $\pm$ 4.18 ^D	163.17 $\pm$ 11.97 ^C	134.00 $\pm$ 8.73 ^B	99.83 $\pm$ 0.95 ^A

Mean with different superscript (A, B, C) vary significantly ($P\leq 0.05$) within the group. Mean with different superscript (a, b) vary significantly ($P\leq 0.05$) in-between the group.

progressively in both the groups. The mean values of pain scores were found to be significantly ($p < 0.05$) different on 0th and 3rd day when compared in between the groups as depicted in Table 1. Baba *et al.* (2012) observed higher pain scores on 3rd day as compared to 0th day values in stray dogs undergoing pinhole castration. Sachin (2015) observed pain scores were significantly higher on 0th day in dogs undergoing surgical as well as laparoscopic cryptorchidectomy. Elevated pain scores in group II might be due to the higher intensity of trauma to tissue. According to Hellyer *et al.* (2007), pain is a typical reaction to tissue injury and inflammation that take place after surgery or tissue injury.

Oxidative stress: There was significant increase in mean values of MDA on 3rd day when compared with 0th day and then decreased significantly ($p < 0.05$) on 14th day and 28th day in both the groups. There was no significant difference observed in MDA and GSH values among the groups as depicted in Table 1. Results of present study were in accordance with Mahalingam *et al.* (2009 and 2014) and Sakundech *et al.* (2020). They observed increase in MDA levels on 3rd day in dogs undergoing castration in male and female dogs. There was significant decrease in mean values of GSH on 3rd day when compared with 0th day and then increased significantly ($p < 0.05$) on 14th day and 28th day in both the groups. There was no significant difference observed when compared in between the groups as presented in Table 1. Results were not in accordance with Mahalingam *et al.* (2009 and 2014) as they observed increase in GSH levels on 3rd day in comparison to preoperative values in dogs undergoing laparoscopic castration, vasectomy as well as conventional castration in male dogs.

Hormonal analysis: The estimation of cortisol (nmol/L) revealed that there was significant ($p < 0.05$) increase on 3rd day in comparison to 0th day and significant decrease ($p < 0.05$) on 14th day and further decreased on 28th day non-significantly ($p > 0.05$) in both the groups. There was no significant difference observed when compared in between the groups as shown in Table 1. Results were in accordance with Okwee-Acai (2012) who observed significantly higher plasma cortisol concentration on 5th day in comparison to 0th, 10th, 15th and 20th days in dogs undergoing pinhole and open surgical castration. Increase in cortisol might be attributable to inflammation and pain due to trauma following surgery as they are major inducers of cortisol secretion (Kehlet 1991). Endocrine system-mediated pain-induced stress reactions are characterised by an increase in cortisol (Hellyer *et al.* 2007). In order to measure stress and pain in dogs, cortisol concentration has been measured (Okwee-Acai *et al.* 2012). There was significant ($p < 0.05$) decline in mean testosterone levels on 3rd day when compared with 0th day and then non-significant decline on 14th and 28th day in both the groups. The mean testosterone levels were significantly higher on 3rd and 14th day in group I as compared to group II. The decline noticed was corresponding to Abou-Ahmed

et al. (2012) after 2 months in donkeys that had undergone pinhole and section ligation and release castration. However, the decrease was more in group II as compared to group I because spermatic cord was incised in group II after ligation. While testosterone levels were not at par and did not differ from each other when compared on remaining time intervals on between the groups. Both the techniques led to substantial decrease in plasma testosterone on 28th day. This might be attributable to that there was successful ligation without revascularization of testicles. Within a few hours, irreversible acute ischemia damage sets in; decreasing the possibility of testicular tissue partial survival and revascularization as a long-term consequence is not expected (Mir *et al.* 2018).

Ultrasonographic examination: On the 0th day the echotexture of dog's testicular parenchyma was homogenous and had a granular pattern in both the groups. The tunics were visible as a thin hyperechoic capsule and there was a linear hyperechoic structure in the central long axis called the mediastinum testis, a landmark for their identification during ultrasonography. These findings were like those of Pugh *et al.* (1990) in dogs. On 3rd day, the testicular parenchyma appeared to be little bit heterogenous along with slight accumulation of peri testicular fluid in both the groups. On 14th day after pinhole and section ligation and release castrated testis the parenchyma appeared to be heterogenous in appearance along with accumulation of more peri testicular fluid. The epididymis also started becoming more echogenic in comparison to testicular parenchyma after castration in both the groups. On 28th day, the testicular parenchyma became extremely heterogenous with hyperechoic spots and there was minimal peri testicular fluid after castration in both the groups. Upon doppler ultrasound, no blood flow was noted in spermatic cord on 3rd, 14th and 28th day after castration (Fig. 3). Sawhney (2016) also observed testicular parenchyma slightly less echogenic with hyperechoic spots/testicular microliths after pinhole castrated bulls. Ahmad *et al.* (1991) and Ahmad and Noakes (1995) observed that testicular parenchyma was not homogeneous and had mottled appearance with a thick echogenic tunica albuginea in small ruminants with testicular degeneration. Pixel values were found significantly ($p < 0.05$) lower on 3rd and 14th day when compared with 0th day in both the groups. There was no significant difference observed in between the groups. The results of present study are in accordance with Arteaga *et al.* (2005) as they also observed decrease in pixel intensity after deleterious effects of scrotal insulation in bulls' testicles leading to decrease in semen quality.

Histopathological examination: Histopathological sections on day 0 from the dog's testes of group I and II revealed normal seminiferous tubules that were lined by intact basal epithelium, germinal epithelial cells and spermatozoa in lumen in both the groups. These results were in accordance with Bhagyalakshmi *et al.* (2020). Histological examination of dog's testis after pinhole castration on 28th postoperative day revealed

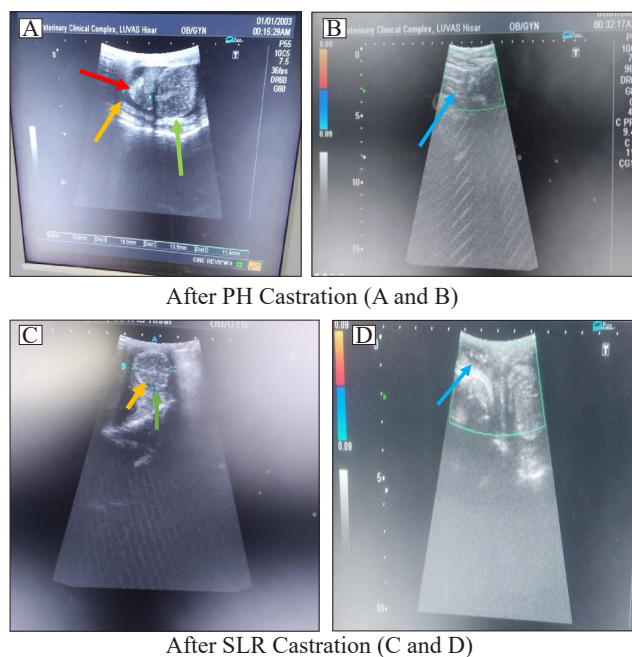


Fig. 3. On 28th day: (A) After PH castration testicular parenchyma became extremely heterogeneous (green arrow) with decreased in peri-testicular fluid (yellow arrow). Epididymis was more echogenic (red arrow) to parenchyma in comparison to previous days of ultrasonographic examination. (B) There was no blood flow noticed in spermatic cord (blue arrow) and testicle (blue dotted arrow) after colour doppler examination. (C) While, after SLR castration, testicular parenchyma became extremely heterogeneous (green arrow) with absence of peri-testicular fluid (yellow arrow) when comparing it to previous days of ultrasonographic examination. Along with increase in echogenicity of epididymis (red arrow) was markedly noticed. (D) On colour doppler examination, blood supply in spermatic cord (blue arrow) was completely absent.

degenerative changes and necrosis in seminiferous tubules that were filled along with homogeneous pink coloured hyalinised material and there was mild infiltration of mononuclear cells mainly lymphocytes in dogs undergone pinhole castration (Fig. 4A). Baba *et al.* (2013) noticed degeneration of spermatogonial cell, hyalinization and atrophy of seminiferous tubules during histopathology in dog's testis, 28th day after pinhole castration. Similar findings were observed by Ponvijay (2007) in calves and Munahi and Abid (2011) in bucks. Okwee-Acai *et al.* (2012) observed coagulative necrosis in dog's testicles 21 days after pinhole castration. On 28th postoperative day, the sections of testis after section ligation and release revealed coagulative necrosis in seminiferous tubules along with accumulation of cellular debris in lumen and surrounded by fibrous connective tissue (Fig. 4B). These findings were similar as that of Abou-Ahmed *et al.* (2012) in donkeys and Saifzadeh *et al.* (2008) in horses after section ligation and release castration.

Surgical time: In animals that had undergone pinhole castration, average surgical time was around 6.11 ± 0.42 min for both the testicles and it was more in comparison to time taken by Fazili *et al.* (2009) and Baba *et al.* (2013) in rams

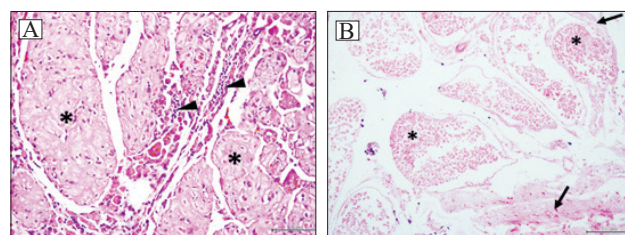


Fig. 4. (A) Photomicrograph of testis on 28th day after PH castration showing degenerative changes and necrosis in seminiferous tubules filled with homogeneous pink coloured hyalinized material (asterisks) and mild infiltration of mononuclear cells mainly lymphocytes (arrow heads). H&E×200; (B) Photomicrograph of testis on 28th day after SLR castration showing coagulative necrosis in seminiferous tubules with accumulation of cellular debris in lumen (asterisks) and surrounded by fibrous connective (arrows). H&E×200.

and dogs, respectively, as both of them did not calculate the mean surgical time and have calculated minimum time required for pinhole castration. In section ligation and release castration, average castration time taken for performing surgery was 16.63 ± 2.03 min and was quite less in comparison to time taken by Abou-Ahmed *et al.* (2012) for section ligation and release castration in donkeys. This difference might be due to larger incision required for exteriorizing the thick spermatic cord of donkeys and suturing it. Very less time was consumed by pinhole castration when compared with section ligation and release castration; these findings were very similar to those of Abou-Ahmed *et al.* (2012) in donkeys.

From the above study, it is concluded that testosterone levels were markedly reduced in both the techniques. Ultrasonographic and histopathological examination revealed marked testicular degeneration in both the techniques. Thus, both castration techniques were equally effective. However, pinhole castration technique is minimally invasive, less time consuming, economical and has less post-operative complications in comparison to section ligation release technique.

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