ISSN: 2456-2912
VET 2024; 9(1): 1088-1090

www.veterinarypaper.com
Received: 16-11-2023
Accepted: 30-12-2023

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Histopathological evaluation of testicles from pin-hole castrated piglets

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Abstract
This study evaluated histopathological changes in the testicles of male piglets that underwent the pinhole method of castration. Animals (n=18) were randomly allotted to 2 groups: group I (pin-hole method of castration) and group II (conventional surgical method of castration). Percutaneous (*in situ*) spermatic cord ligation was performed under local anaesthesia in all the piglets of group I. After pinhole castration, the testicular dimensions of piglets in Group I were measured on days 0, 14, and 28. On the 28th post-pinhole castration day, bilateral orchiectomy was performed, and both testicles were examined for histopathological studies. Histopathological examination revealed testicular degeneration and ischemic coagulative necrosis and thus confirmed testicular dysfunction.

Keywords: Histopathology, Testicles, Piglets, Pinhole castration.

Introduction
Castration in piglets is a routine activity in pig farms to calm down the aggressive behaviour of males, to avoid unwanted breeding, and to prevent unpleasant odour from the cooked meat or boar taint or meat taint caused by the accumulation of 16-androsterone and skatole in the fat of uncastrated male piglets. Most pig farms in India and around the world use the conventional surgical method of castration. An alternative method called the 'pinhole castration technique' has recently been found suitable in calves, kids, and dogs (Ponvijay, 2007; Okwee-Acai *et al.*, 2008; Fazili *et al.*, 2009; Baba *et al.*, 2013) [13, 12, 5, 3] because this technique is less invasive, cheaper, easier, and faster than conventional surgical castration. In this context, a histopathological study was planned to evaluate the pinhole castration technique in male piglets.

Materials and Methods
The study was performed on 18 apparently healthy Large White Yorkshire crossbred piglets aged one to two months with an approximate weight of less than 10 kilograms, raised at the Centre for Pig Production and Research, Mannuthy, who were selected for the study.
Animals were assigned randomly to either pinhole castration groups (12 animals), designated as Group I, or the conventional surgical method of castration (6 animals) as Group II. All the piglets were weighed, and detailed physical and clinical examinations were conducted before the actual start of the study. The pinhole method of castration in piglets was carried out by the procedure described by Ponvijay (2007) [13] in dogs, and the conventional surgical method of castration was performed in group II (Haga and Ranheim, 2005; Hofmann *et al.*, 2019; Fredriksen and Nafstad, 2006; Von Borell *et al.*, 2009) [10, 11, 6, 17].
Body weight and scrotal dimensions were measured to assess overall growth and changes in the testicles of piglets undergoing pinhole castration. In Group I, both parameters were measured on days 0, 14, and 28 following spermatic cord ligations.
The testes, epididymis, and spermatic cord were removed under local anaesthesia on the 28th day after pinhole castration using the open-covered method of castration (Von Borell *et al.*, 2009; Hofmann *et al.*, 2019) [11, 17], and histopathological changes were examined. For light

microscopy, testicular samples were immediately fixed in 10% neutral phosphate-buffered formalin for 24 hours and then embedded in paraffin.

Samples were cut using a microtone into sections/slices of approximately 5µ thickness and afterwards stained with haematoxylin and eosin (H&E). The slides were examined using a Leica-Dm 200-led trinocular research microscope (Nussloch, Germany), as described by Suvarna *et al.* (2018) [16].

Results were analysed by using two-way repeated measures ANOVA between the groups and within the group during the study period and expressed as the mean standard error of the mean.

Results and Discussions

Body weight and scrotal dimensions

The body weight of piglets (Mean ± SE) before and after the treatment in groups I and II ranged between 9.38 kg to 11.72 kg and 9.58 kg to 11.56 kg. On day 28, it was noted that piglets who had undergone pinhole castration had significantly higher mean body weight ($p \leq 0.05$) than those who had undergone standard surgical castration.

At different time intervals, the mean length of both testicles ranged between 4.89 cm and 3.33 cm, and the mean width of both testicles ranged between 3.69 cm and 2.30 cm. Higher values were noted on day 14 than on day 0, and lesser values were recorded on day 28 of post-pinhole castration (Table 1). Mean ± S. D values of testicular dimensions (length and width) of Group I at various time intervals have been presented in Table No. 1.

Table 1: Mean ± S. D changes in testicular length and width of treatment Group I

Parameter	0 day (Base value)	Day 14	Day 28
Testicular length (cm)	4.89± 0.15*	6.77± 0.32*	3.33±0.27*
Testicular width (cm)	3.69 ± 0.39*	4.13± 0.06*	2.3±0.67*

(*Indicates significant difference from the base value within the group ($p<0.05$)

Histopathological Examination

In group I, an orchiectomy was done after twenty-eight days of pinhole castration in all the piglets, and testicular samples were studied for histopathology. According to Dixit (1977), vascular blockage to the testis for twelve days caused the replacement of seminiferous tubules by amorphous masses devoid of the basement membrane and caused atrophy of Leydig cells. In the present study, spermatic cords were ligated for twenty to eight days, which was more than enough to cause testicular shrinkage and complete ischemic necrosis.

The pinhole technique revealed testicular degeneration, atrophy, acute ischemic coagulative necrosis of germinal epithelium, blood vessel congestion, inflammatory cell infiltration, and seminiferous tubule hyalinisation. (Fig. 1 to Fig. 4). Similar findings were reported in previous studies of pinhole castration in bull calves, goats, and dogs (Ponvijay, 2007; Okwee-Acai *et al*., 2008; Fazil *et al*., 2013; Baba *et al*., 2013) [13, 12, 3]. Bergh *et al.* (2001) [2] stated that severe ischemia kills all parts of the testis. It has been reported that testicular degeneration and atrophy lead to testicular dysfunction (Awal *et al*., 2004) [1].

There was significant interstitial fibrosis and degenerative and necrotic changes in Sertoli and Leydig cells. Smith (1955) [15] reported that stoppage of testicular blood flow more than four hours resulted in the cessation of spermatogenesis as well as damage to the Sertoli and Leydig cells, while ten hours or more of sustained ischemia resulted in the total elimination of Leydig cells and the replacement of testicular components with fibrous connective tissue.

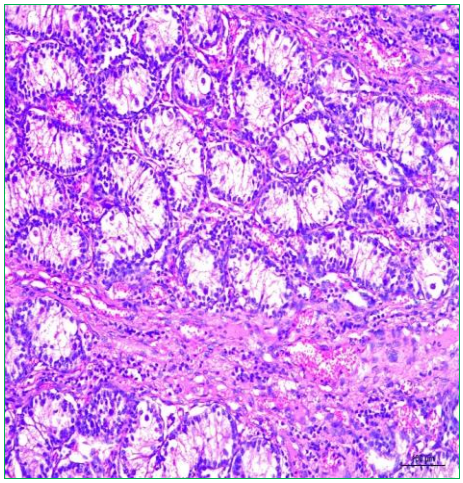

Fig 1: Infiltration of neutrophils in interstitium and necrosis of cells (H&E 100X)

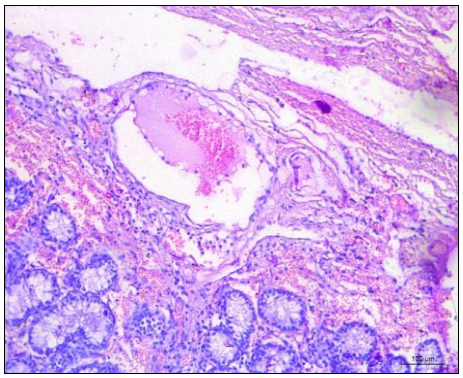

Fig 2: Congestion of blood vessels and infiltration of inflammatory cells in the interstitial cells (H&E 100X)

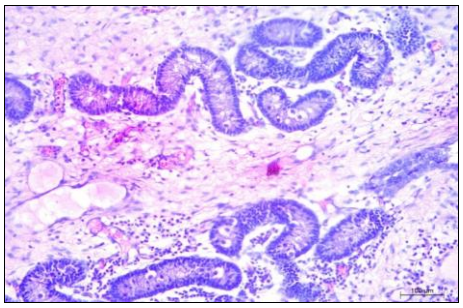

Fig 3: Neutrophilic infiltration in seminiferous tubules and interstitium (H&E 100X)

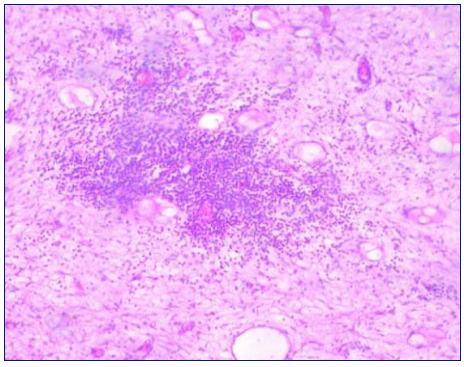

Fig 4: Severe infiltration of inflammatory cells (H&E 100X)

In the same study, testes from the piglets subjected to the conventional surgical method were collected and examined for normal histomorphology. The histopathological finding of the testicles revealed normal structures like tunica albuginea, trabeculae mediastinum testis, seminiferous tubules, and interstitial tissue (Fig. 5). The connective tissue trabeculae extended from the capsule and divided the parenchyma of the testis into several lobules. The seminiferous tubules contributed to the major portion of testicular parenchyma, with each seminiferous tubule comprising outer lamina propria and seminiferous epithelium. Similar findings were reported by Gaykee *et al.* (2008) [8] in Neelgai, Yaseen (2009) [18] in goats, Gleide *et al.* (2010) [9] and Singh *et al.* (2019) [14] in pigs.

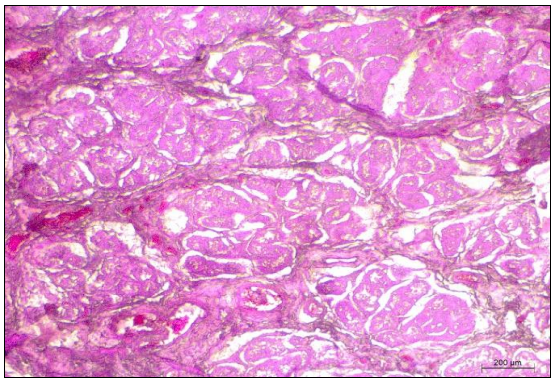

Fig 5: Normal histology of seminiferous tubules and interstitium (H&E 200X)

Based on the findings of the present study, it was concluded that pinhole castration caused testicular dysfunction and atrophy.

Summary
In piglets, the pinhole castration method can be performed by ligating the spermatic cord in situ, resulting in testicle atrophy and permanent dysfunction of the testicles. The histopathology finding of the testicles from the piglets subjected to the pinhole technique revealed testicular degeneration and atrophy, a typical condition that leads to testicular dysfunction. The study concluded that the pinhole castration technique was advantageous in piglets because it was a minimally invasive, simple, and alternative method for piglet castration.

Authors' declaration of interests: No conflicts of interest have been declared.

Source of funding: None.

Acknowledgement
The authors are highly grateful to the Professor and Head of the Centre for Pig Production and Research, Mannuthy, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, for providing the necessary facilities and encouragement to conduct this study.

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