



Effect of Vasectomy on the Testosterone Levels and Testicular Structures in Bucks for Long Term

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ABSTRACT

This study was conducted on 12 adults local crossbreed Bucks to investigate the long-term effect of the vasectomy on Testosterone levels, sexual efficiency, and testicular structures. The bucks were divided into 4 groups according to the duration after the surgery. Following surgery, testosterone hormonal fluctuations were observed in association with stressed factors such as operation pains. Initially, surgical discomfort increased sexual arousal time during the first week post-operation. As healing progressed, arousal time gradually declined by the fourth week. However, ejaculate volume showed a continuous decline throughout the study. A similar pattern of decline, followed by an absolute lack, is noted in sperm motility in all the bucks by the end of the fourth week. Testosterone levels evaluated by enzyme-linked immunosorbent assay (ELISA), demonstrated fluctuations. Post operation testicular parenchyma sections during the first month indicated injury to seminiferous tubules due to increase in the count of Leydig cells. By the second month tubules had separated and shrunken, accompanied by a decline in Leydig cells. During the 3rd month, the seminiferous tubules disappeared, and the interstitial tissue extended with renewed proliferation of the Leydig cells. However, by the fourth month, the interstitial tissue suffered from mild shrinkage, and Leydig apoptosis were evident. In conclusion, vasectomized bucks demonstrated the suitability to use the males for teasers at the first period. But the libido declined during the second period due to the scrotal pains. Then these pains became chronic and less severe in the third month, which improved the sexual desire.

Keywords

*Leydig cells; oxidative stress; Seminiferous tubules; Sertoli cells;
Sexual desire; Spermatogonia; Vasectomised buck.*

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Abbreviations

SPSS: Statistical Package for Social Sciences
M±SE: Standard Error.
ANOVA: Analysis of Variance.
LSD: Least significant difference.

Introduction

Vasectomy is a common and reliable method of male contraception; however, the impact of the surgical intervention on sexual efficiency and libido disorders is unknown [1]. Testosterone is the hormone responsible for libido and overall sexual behaviour in males [2].

Testosterone hormone effects on the brain by neurohormonal pathway, modulating neurotransmitter activity and influencing several brain functions and behaviors. Moreover, androgen hormones modify different stages in neurogenesis of adult, by induced alterations at neurogenesis, as well as the steroids selectively improve the maintenance of recently generated nerve cells, although they have simple influence on cell proliferation [3].

Testosterone-secreting Leydig cells are derived from peritubular stem cells, which transform into spindle-shaped progenitor cells. Once they proliferate and differentiate into immature cells, their cytoplasm contains numerous droplets with a small amount of Testosterone. Immature Leydig cells eventually differentiate into adult cells, which contain the androgen hormones, mainly the testosterone hormone [4].

So far, the research that highlights the relationship between sexual behaviour and testicular structure in vasectomized bucks for a long term is severely lacking [5]. To narrow this gap, the current study was designed to examine the long-term association among the testosterone levels, sexual efficiency and testicular changes in vasectomized bucks.

Result

Sexual Assessment

Sexual behavior evaluation revealed that sexual arousal time increased during the first week post-operation in all bucks. This was followed by a gradual decline until the fourth week, when the values approached the level prior to operation, returning to nearly normal level. Conversely, ejaculate volume showed a decline until the last weeks of evaluation. In all the vasectomised males, sperm was absent in the ejaculate samples, as a result of sperms retention at epididymis. Microscopic examination showed a reduction in the percentage of sperm motility, eventually sperm motility had disappeared by the third and fourth periods in all bucks, as summarized in Table 1.

The different superscript letters (a, b, c, d) within columns show statistically significant differences ($p < 0.05$).

Testosterone Assessment

Testosterone levels appeared to be elevated during the first month post vasectomy, compared with the control and zero period (pre-vasectomy) values. However, in the second month testosterone levels significantly dropped ($p < 0.05$), in contrast to the control group. By the third month, levels elevate again, al-

Table 1. The sexual assessment appeared weeks prior and post vasectomy operations.

Week	Desire time / minute	Ejaculate volume/ ml	Individual motility %
Zero	2.25 ± 0.5 ^a	0.752 ± 0.1 ^a	5.00 ± 2.04 ^a
1 st	9.24 ± 0.5 ^c	0.31 ± 0.09 ^b	36.25 ± 3.4 ^b
2 nd	4.42 ± 0.4 ^b	0.25 ± 0.08 ^b	1.55 ± 1.2 ^c
3 rd	3.35 ± 0.6 ^a	0.23 ± 0.07 ^b	0.00 ^d
4 th	2.2 ± 0.2 ^a	0.21 ± 0.07 ^b	0.00 ^d

The different superscript letters (a, b, c, d) within columns show statistically significant differences ($p < 0.05$).

though the increase was not significant. In the fourth month, testosterone levels decreased once more, showing a significant difference ($p < 0.05$) compared with the zero period, as summarized in Table 2.

The different superscript letters (a, b) within the row show statistically significant differences ($p < 0.05$).

Histopathological Assessment

Histological evaluation of vasectomised tests at the first month post-operation revealed degradation of the seminiferous epithelium with apoptosis among spermatocytes, spermatids and Sertoli cells. A small portion of the spermatogonia of tubules were observed separating from the germinal layer and migrating toward the lumen. On the other hand, the interstitial tissue size increased, likely due to the proliferation of Leydig cells, at the expense of shrinking seminiferous tubules (Figure 1).

By the second month, all seminiferous tubules appeared shrunken and separated from the interstitial tissue toward the central lumen, with nearly all their cells undergoing apoptosis. The interstitial tissue size and Leydig cells population also decreased, resulting in prominent empty spaces between degenerated tubules (Figure 2).

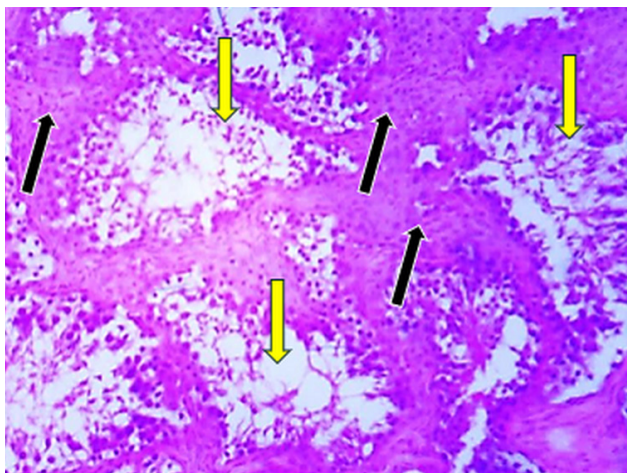
In the third month, the pattern shifted again. Due to the proliferation of Leydig cells, the interstitial tissue completely disappeared from the seminiferous tubules, and the interstitial tissue formed distinct islands surrounded by the empty spaces (Figure 3).

During the fourth month, the histopathological features were similar to the third month, but with

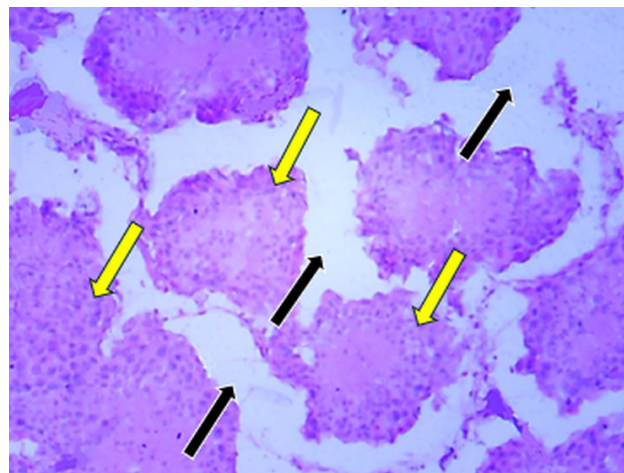
Table 2.

. The effects of testosterone levels at prior and post vasectomy operations.

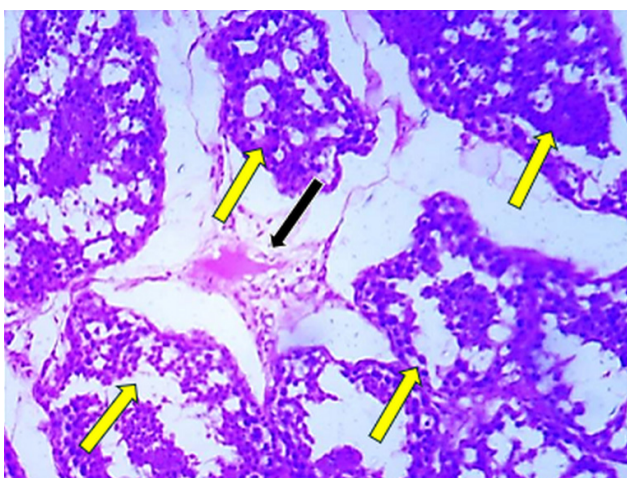
Group/Month	Zero	1st	2nd	3rd	4th	LSD
Testosterone level (pg/ml)	180.2 ± 20.13 ^a	186.03 ± 19.28 ^a	149.27 ± 15.5 ^b	187.5 ± 23.6 ^a	143.77 ± 13.77 ^b	169.4 ± 8.63

The different superscript letters (a, b) within the row show statistically significant differences ($p < 0.05$).**Figure 1.**

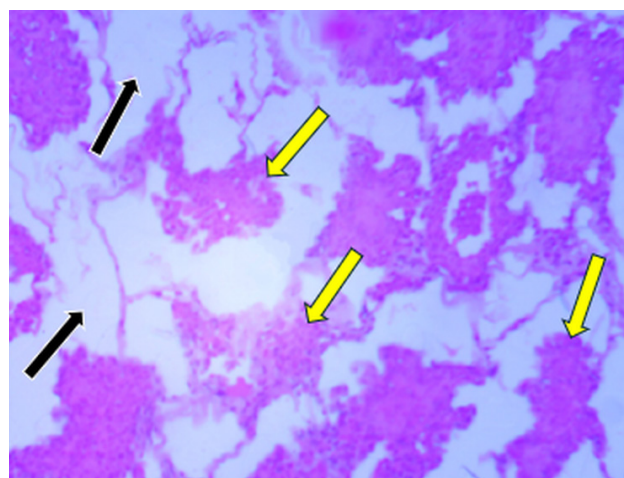
Parenchymal tests at first month post vasectomy showed destruction of the seminiferous epithelium and their cells suffering from apoptosis, and tubules are initiated to separate from the germinal layer and pushed toward their lumen (yellow arrows). The interstitial tissue size increased, with proliferation of Leydig cells (black arrows).

**Figure 3.**

Parenchymal tests at third month post vasectomy expanded the interstitial tissue with proliferation of Leydig cells (yellow arrows), also disappeared the seminiferous tubules features completely (black arrows).

**Figure 2.**

. Parenchymal tests at second month post vasectomy appeared the seminiferous tubules were separated and shrank toward the central lumen, and all their cells suffering from apoptosis (yellow arrows), with decreased the interstitial tissue size and Leydig cells account (black arrow).

**Figure 4.**

Parenchymal tests at fourth month post vasectomy expanded mild shrinkage of the interstitial tissue and some of Leydig cells having undergone apoptosis (yellow arrows), with extended the empty spaces among the islands of interstitial tissues (black arrows).

minor differences. Slight shrinkage of the interstitial tissue and mild apoptosis among Leydig cells were observed, accompanied by more extensive empty spaces surrounding the islands of interstitial tissues (Figure 4).

Leydig cell counts was recorded. The average number of Leydig cells among three seminiferous tubules was 45.39 ± 1.4 in the first month. This number

decreased to 36.25 ± 0.8 in the second month, then returned to 47.33 ± 1.3 in the third month. By the fourth month, Leydig cell numbers decreased significantly ($p < 0.05$) compared with the first month, as shown in Table 3.

The different superscript letters (a, b) within the row show statistically significant differences ($p < 0.05$).

Table 3.
The leydig cells count at post vasectomy operations

Group/Month	1st	2nd	3rd	4th	LSD
Leydig No.	45.39 ± 1.4 ^a	36.25 ± 0.8 ^b	47.33 ± 1.3 ^a	33.03 ± 1.3 ^b	40.5 ± 0.3

The different superscript letters (a, b) within the row show statistically significant differences (*p* <0.05).

Discussion

The results of this study demonstrate that, sexual arousal time in bucks decreases during the first week following vasectomy, most likely due to postoperative pain associated with the surgical procedure . The arousal time gradually decreased in the later weeks, until it returned to normal levels (similar to the pre-operation levels) by the fourth week. Similar observations were reported by several researchers, such as [9]. As a result [10, 11] and [12], who stated that pain may persist for up to one week post operation, before gradually decreases in later weeks. Therefore, the vasectomised bucks should be given resting period of 2-3 weeks before being used as teasers. This period allows complete recovery from stress and psychological factors associated with surgical operation such as anesthesia administration, incision, dissection of sensitive tissue and the pain that can continue for several days after the operation [13].

The ejaculate volume showed a significant (*p*<0.05) decline beginning in the first week post-vasectomy operation until the fourth week, in contrast to its value prior operation. This reduction can be attributed to the occlusion of the reproductive tract, which prevents the testicular secretions, although a small amount of ejaculate remained likely originated from the accessory sex glands outside the testicles. This confirms the success of this surgical technique in inducing infertility [14, 15].

Microscopic examination of sperm individual motility of semen revealed a decrease of sperm percentage during the few weeks after operation, ultimately reaching complete stop and disappearance by the third and fourth weeks. This loss of sperm motility is a direct consequence of the sperm transport disruption. This data was also provided by [13].

Post vasectomy, the levels of testosterone in bucks were estimated and were found to be at zero

group level (prior vasectomy); the vasectomy did not influence the secreted testosterone and sexual desire during the first month post vasectomy as a result of unaffected of interstitial cells and tissue by occluded passages [14]. However, by the second month, a notable decline in testosterone hormone was observed. This reduction may be explained by the scrotal pain resulting from semen accumulation within the epididymal duct and its enlargement [16].

This condition is similar to the chronic post-vasectomy pain syndrome described in human, which can be treated in this state by suppuration of the scrotum and administration of non-steroidal anti-inflammatory drugs (NSAIDs) as the first line of treatment. In the event of the treatment failure, caudal epididymectomy may be required [17,18]. By the third month post-vasectomy, the hormonal concentration rebounded to the higher levels, likely due to two factors: first, the reduction of severe scrotal pain with the transformation of acute scrotal pain into chronic scrotal pain, resulting from rupture of the epididymal and vas deferens ducts proximal to the ligature site, and exudation of semen into the surrounding tissue and formation of the sperm granulomas [19,20,21]. Second, the proliferation of Leydig cells within the expanded interstitial tissue, which secretes testosterone at the expense of the degraded seminiferous tubules [15,22]. However, during the fourth month, testosterone values declined as interstitial tissue shrank and a reduction of Leydig cell quantities, which are endocrine units of the testosterone hormone [23].

Parenchymal tests in the first month post vasectomy showed extensive destruction of the seminiferous epithelium and its cells (Spermatogenesis and Sertoli cells) undergoing apoptosis. These structures were separated from the germinal layer and pushed toward their lumen, caused due to the elevation of intraluminal pressure be-

cause of the vas deferens ligature. Although the interstitial tissue increased in size, and has shown proliferation of Leydig cells [24]. By the second month, the tubules were separated and shrank toward the central lumen, nearly all cells were apoptotic [22]. Decreased interstitial tissue size and Leydig cells accounted for the long-term vasectomy impact on the modulation of macrophage pathways, which regulate testicular immuno-endocrine functions [25]. During the third month, the number of Leydig cells increased again, similar to levels observed in the first month due to expansion of interstitial tissue and proliferation of Leydig cells. However, by the fourth month, interstitial tissue size retracted, and Leydig cell populations reduced, rendering the structures useless and exposed these cells for apoptosis [26,27].

In conclusion, the findings from this study demonstrated the suitability of using the males for teasers only during the first month. Libido declined during the second month, due to scrotal pains; thus, animals at this stage should be rested or considered for caudal epididymis removal surgery. These pains decreased during the third month to transform from the acute to chronic pain, which improved the sexual desire. By the fourth month, libido weakened again, likely due to Leydig cell degeneration and corresponding with low levels of testosterone.

Materials and Methods

Ethical Approval

All experimental procedures were conducted at the Faculty of Veterinary Medicine farm, University of Kufa, under the care and supervision of a veterinarian. Also the study was approved by the Scientific Ethic Committee, Faculty of Veterinary Medicine, University of Kufa (Approval No: 2117).

Experimental Animals

The study was conducted on 12 adult local cross breed Bucks aged between 1-2 years, and weighting 30-45 kg. The animals were acclimatized and observed at the Faculty of Veterinary Medicine, University of Kufa, from February 2023 to June 2023. All bucks were maintained under uniform nutritional conditions throughout the study. The feeding regimen consisted of 25% concentrated food (50% corn, barley, with 50% bran, soybean and supplement) and 75% hay, with free access to salt blocks and water, since 2 weeks before study until experimental end. All Bucks applied for Vasectomy operation were divided into 4 groups based on the period of post-operation: the first group continuing for one-month post-vasectomy, the second group for two months, the third group for three months and the fourth group until four months. The ex-

perimental timeline extended March 2023 to June 2023.

Vasectomy Procedure

The scrotal coats of each buck was clipped, shaved, and cleansed with soap followed by a 70% alcohol antiseptic solution. Sedation was achieved with Xylazine 2% (Alfasan, Woerden, Holland) administered intramuscularly at 0.2 mg/kg body weight (B.W). Local infiltration anesthesia was provided using lidocaine 2% (Rotexmedica, Trittau, Germany) at a dose of 4 mg/Kg B.W administered subcutaneously. The caudal aspect of the para-median scrotal skin was incised about 2 cm at the scrotal neck level on both sides. Also, the underlying tunica vaginalis was incised. The ductus deferentes were elevated with curved forceps and were double ligated by absorbable synthetic suture material (#0), spaced 1 cm apart, and 0.5 cm segment between the ligatures were excised. The ligated ends were returned to their ligature ends. The incised skin was sutured by a simple interrupted suture pattern using non-absorbable synthetic suture material (#1) monofilament polyamide. Another ductus difference was made in a similar manner, following the method described by [5].

Sexual Assessment

Ejaculate were collected from each buck using artificial vagina into a warmed glass tube at 37°C prior to vasectomy to evaluate the sexual efficacy. Sexual arousal time was measured from the duration of exposure of the female to the adult male until the mating moment and is recorded in minutes. Ejaculate volume was documented using a graduated glass tube (ml). Sperm motility was evaluated on the warmed slide (37°C) under electronic microscope (×400). The percentage of progressive sperm movement, was estimated visually on a 0-100% scale [6]. These investigations were performed on each Buck before the operation as a normal grade (considered the 0 or control period), and subsequently at 4, 7, 10, 14, and 21 days post-operation.

Testosterone Assessment

Blood samples (5 ml) were collected using from the jugular vein of each buck in the morning hours. Samples were placed in sterile transport tubes to isolate serum, and kept at -20 °C until analysis. Serum testosterone levels was measured using an ELISA kits (Goat Testosterone ELISA Kit "HSD17B3 elisa kit" from MyBioSource.com Germany) with Infitek Mpr-H200bc Elisa Machine Microplate Reader with 96/48 -Well Plate [7]. Blood samples were collected at (0) period as a normal grade (control) prior to operation, and at 1, 2, 3, and 4 months post-operation.

Histopathological Assessment

For histological evaluation, testicular biopsies (1cm³) were obtained after castration at 1, 2, 3 and 4 months post-operation, for 1, 2, 3 and 4 groups, respectively. Biopsies were fixed in 10% neutral buffered formalin for 72 hours, dehydrated, cleared in xylene and embedded in paraffin wax using routine methods. Sections of 5–6 µm thickness were cut, de-waxed, cleared in xylene, rehydrated and stained with Hematoxylin and Eosin for light microscope examination. Leydig cells were counted following the method described by [8], the number of Leydig cells among the three seminiferous tubules. A total of 20 areas for each section (slide) were examined, and these areas were distributed four in each corner and the last four in the centre of the slide.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 25). All data were expressed as Mean ± Standard Error (M ± SE), and differences

among the groups of animals were compared using one-way Analysis of Variance (ANOVA). The least significant difference (LSD) was used to significantly compare between means for simplest and limited the samples. The level $P < 0.05$ is considered to be significant.

Authors' Contributions

Hussein K and Dhurgham H participated in the study of conception and design. Hussein K: Acquisition of data. Dhurgham H: Analysis and interpretation of data. Hussein K: Drafted of the manuscript. Both authors critically revised the manuscript for important intellectual content and approved the final manuscript.

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Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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