Research Article

Title

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

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Keywords

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

Abstract

This study included twelve adult local breed Bucks to investigate the long-term effect of the vasectomy technique on the Testosterone levels, sexual efficiency, and testicular structures. The bucks were divided into 4 groups according to the duration after the surgery. Vasectomy induces testosterone hormonal fluctuations in males due to the stressed factors such as operation pains immediately after surgery then removed pain by healing and returned to appears as scrotal pain because the pressure of sperms accumulation in occluded sexual passages. Therefore the duration of sexual desire shown an increase after the first week post-operation among all the study groups it declined until the fourth week. Furthermore, the ejaculate volume consistently showed a decrease until the end of 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, all these

data proved the effectiveness of this technique to avoid the sperms pass. Testosterone levels evaluated by enzyme-linked immunosorbent assay kits revealed fluctuations. Post operation testicular parenchyma sections in group 1 indicated injury to seminiferous tubules due to increase in the count of Leydig cells in group 1, while at 2nd month was separated and shrank that tubule, and decreased the Leydig cells account, in 3rd group was observed the seminiferous tubules disappeared, and extended the interstitial tissues with increased the Leydig cells number. In the 4th month, the interstitial tissue suffered from mild shrinkage, with the Leydig cells accounting for it. It is concluded that the vasectomised 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 was dropped in the third month due to its chronicity, which improved the sexual desire.

Abbreviations

SPSS: Statistical Package for Social Sciences

M±SE: Standard Error.

ANOVA: Analysis of Variance.

LSD: Least significant difference.

Introduction

Vasectomy is a routine and reliable way of contraception in males; 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. This can change neurotransmitter action and affecting several functions of brain and behaviors. Also the 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 having simple influence at proliferation of cell [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 vasectomised 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 vasectomised bucks.

Results

Sexual Assessment

Sexual arousal time recorded has shown raised levels in the first week post-operation in all animals, then it decreased gradually until the 4th week, and the values were nearly at the level prior to operation (normal rate). But the ejaculate volume is documented as a whole, a decline was observed until the last weeks of evaluation, where it stabilised in all the vasectomised males

with absence of sperm at ejaculate sample as a result of sperms retention at epididymis. Also, the percentage of sperm motility appeared to be weak during the microscopic examination until it disappeared at the 3rd and 4th periods in all bucks in Table 1.

Week	Desire time /minute	Ejaculate volume/ml	Individual motility %
Zero	2.25±0.5^{a}	0.752±0.1^{a}	85.00±2.04^{a}
1st	9.24±0.5^{c}	0.31±0.09^{b}	36.25±3.4^{b}
2nd	4.42±0.4^{b}	0.25±0.08^{b}	1.55±1.2^{c}
3rd	3.35±0.6^{a}	0.23±0.07^{b}	0.00^{d}
4th	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 ($p < 0.05$).

Testosterone Assessment

Testosterone levels appeared to be elevated in the first period (one month post vasectomy) in all animals compared with the control or zero period (prior vasectomy). But they significantly dropped in the second period ($p<0.05$), in contrast to the control group, and then rebounded to elevate in the third group without a significant difference. While in the fourth period, they decreased with a significant difference ($p<0.05$) compared with the zero period in Table 2.

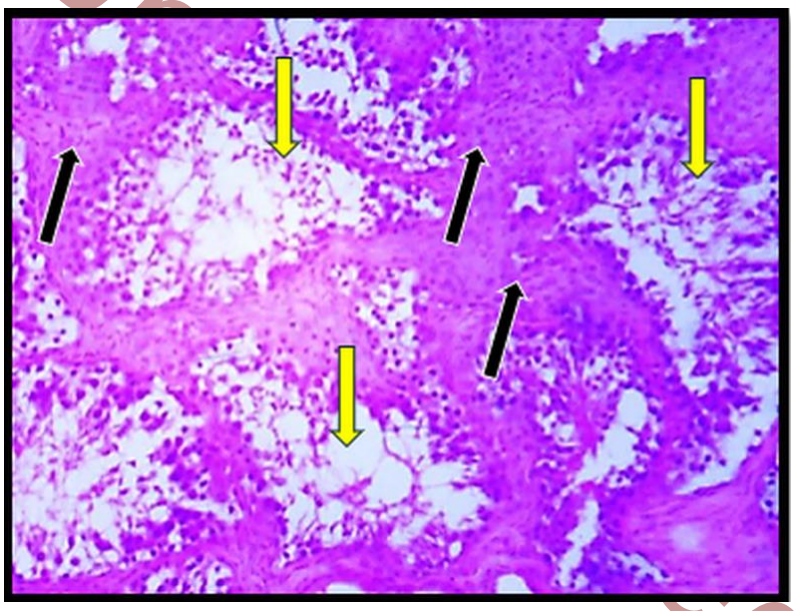
Group/Month	Zero	1st	2nd	3rd	4th	LSD
Testosterone level (pg/ml)	180.2±20.13^{a}	186.03±19.28^{a}	149.27±15.5^{b}	187.5±23.6^{a}	143.77±13.77^{b}	169.4±8.63

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

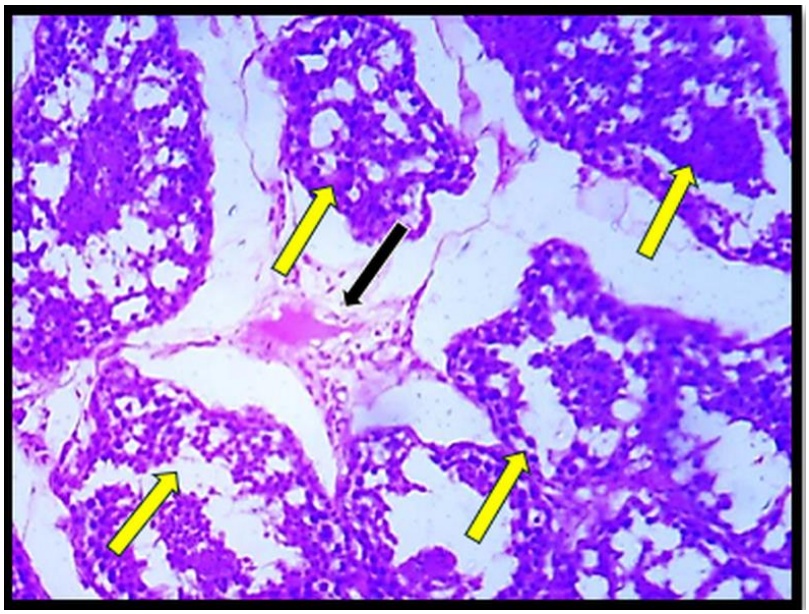
Histopathological Assessment

The histological assessment of vasectomised tests at the first month post-operation revealed degradation of the seminiferous epithelium as well as the spermatocytes, spermatids and Sertoli

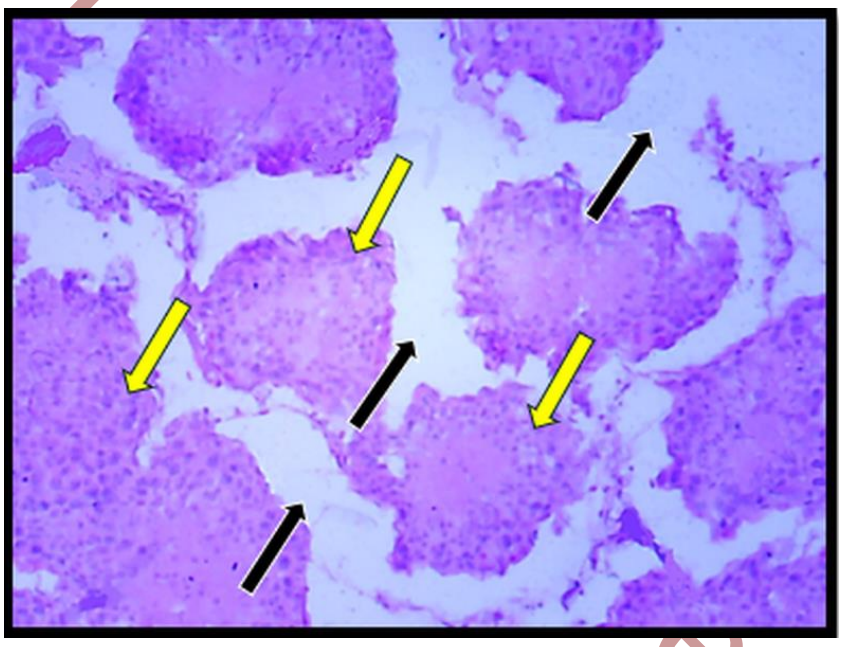
cells affected by apoptosis. A small portion of the spermatogonia of tubules were initiated to separate from the germinal layer and urged toward their lumen. On the other hand, the interstitial tissue size increased at the expense of shrinking seminiferous tubules, attributable to the proliferation of Leydig cells (Figure 1).



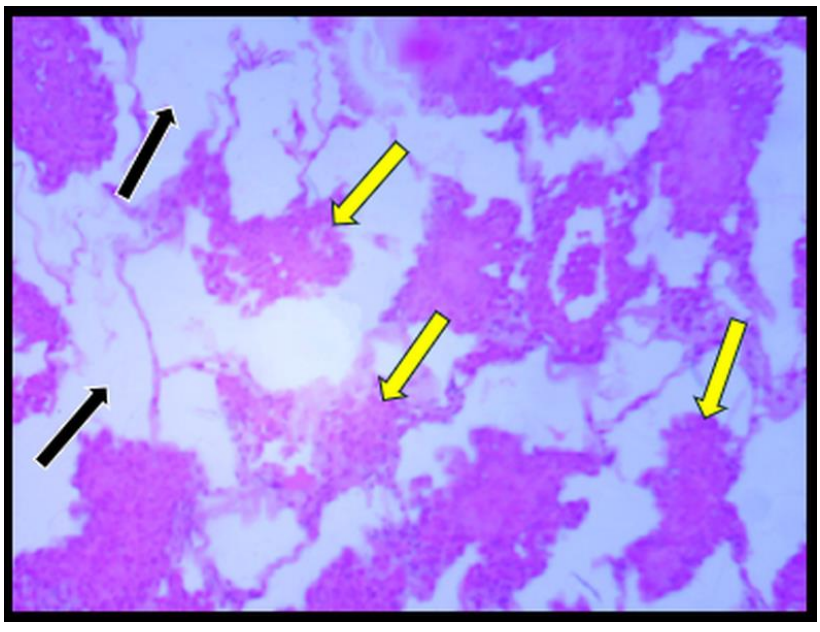
In the second month, all seminiferous tubules were separated and shrank from the interstitial tissue toward the central lumen, and all their cells were affected by apoptosis. A decrease in the interstitial tissue size and Leydig cells caused the empty spaces observed (Figure 2).



However, in the third month, the interstitial tissue due to the proliferation of Leydig cells disappeared from the seminiferous tubules completely, therefore, the interstitial tissue showed as islands circumscribed by the empty spaces (Figure 3).



While in the fourth month, the histopathological features were similar to the third month, but with minor differences such as mild shrinkage of the interstitial tissue and some Leydig cells undergoing apoptosis, with the extended empty spaces among the islands of interstitial tissues (Figure 4).



The Leydig cell count was recorded (45.39±1.4) in the interstitial tissue among three seminiferous tubules of the first group. This count dropped to (36.25±0.8) in the second period and returned to (47.33±1.3) in the third period, and the fourth group documented a significant difference at $p<0.05$ in contrast with the first period shown in Table 3.

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 sexual arousal time decreases in the first week postoperative vasectomy in all bucks owing to the pain of surgery. Therefore, this sign initiated decrease in the later weeks, until it rebounds to normal levels in the fourth week, analogous to the level of arousal time for males' prior operation. The same has been mentioned by several researchers, such as [9]. As a result [10, 11] and [12] stated that the pain continues for one week post-surgical operation and gradually drops in later weeks. Therefore, the vasectomised bucks should be allowed to rest for 2-3 weeks prior to using as teasers to allow for complete healing from stress and psychological factors of surgical section included anesthesia administration, incision, dissection of sensitive tissue and continued the pain for several days post operation [13].

The ejaculate volume decreased in the first week post-vasectomy operation until the fourth week, with a significant difference ($p<0.05$) in contrast to its value prior operation. These developments occurred by occluding the sexual passage and stopped the testicular secretions, although a small amount of ejaculate remained from the accessory sex glands outside the testicles, which led to the success of this surgical technique in inducing infertility [14] and [15].

Likewise, the microscopic examination of sperm individual motility resulted in a decrease of sperm percentage in the ejaculated semen for vasectomised bucks at a few weeks after operation until complete stopped and disappearance of sperms, because 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 in the first month post vasectomy as a result of unaffected of interstitial cells and tissue by occluded passages[14]. In our study, a diminution of testosterone hormone has occurred in the vasectomised bucks in the second month. This may be explained by the scrotal pain due to the accumulation of semen into the epididymal duct and its enlargement [16].
The same was reported in a vasectomised human, referred to as "chronic post-vasectomy pain syndrome", which can be treated in this state by suppuration of the scrotum and administration of NSAIDs as the first line of treatment. In the event of the treatment failure, caudal epididymectomy is to be applied [17,18]. Moreover, the hormonal concentration rebounded to the higher levels in the third month mainly due to two factors: firstly, the reduction of severe scrotal pain with the transformation of acute scrotal pain into chronic scrotal pain as a result of the ruptured sexual ducts (epididymis and vas deferens) before the ligature site and exudation of semen into the surrounding tissue and formation of the sperm granulomas [19,20,21]. Secondly, the expansibility of interstitial tissue with the proliferation of Leydig cells, which secretes testosterone at the expense of the degraded seminiferous tubules [15,22]. Although the testosterone values declined in the fourth period as interstitial tissue shrank and a reduction of Leydig cell quantities, which are endocrine units of the testosterone hormone [23].

The 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 because of the vas deferens ligature. Although the interstitial tissue increased in size, and has shown proliferation of Leydig cells [24]. In the second month post-vasectomy, the tubules were separated and shrank toward the central lumen, with all the cells undergoing apoptosis [22]. Decreased interstitial tissue size and Leydig cells accounted for the long-term vasectomy impact on the modulation of macrophage pathways that regulate testicular immuno-endocrine functions [25]. The increase in Leydig cells levels at the third period were similar to those at first period due to expansion of interstitial tissue with proliferation of Leydig cells, but the group that experienced period four has shown retraction of their size followed by reduction of Leydig cells rendering the structures useless and exposed these cells for apoptosis [26,27].

In conclusion, the first period of vasectomised bucks demonstrated the suitability of using the males for teasers only at first month. Libido declined during the second month, attributable to the scrotal pains therefore should be given rest for males at this period or performed the caudal epididymis removal surgery. These pains decreased during the third month to transform from the acute to chronic pain, which improved the sexual desire. Lastly, the libido of the vasectomised buck at the fourth month was weak as a result of the degenerated leydig cells with low levels of testosterone.

Materials and Methods

Ethical Approval

The work was applied at the Faculty of Veterinary Medicine farm under the care and supervision of a veterinarian, also approved by Kufa University's Faculty of Veterinary Medicine's Scientific Ethic Committee (No: 2117).

Experimental Animals

The current study uses 12 adult local breed (cross breed) Bucks aged 1-2 years old, and their weights were 30-45 kg. They were acclimatised and observed at the Faculty of Veterinary Medicine - University of Kufa, for period from Feb 2023 to Jun 2023, the nutrition continued without vagaries was 25% concentrated food (50% corn, barley, with 50% bran, soya and supplement) and 75% hay with unrestricted access to salty blocks and water, since 2 weeks before study until experimental end. All Bucks applied for Vasectomy operation were categorised into 4 groups based on the period of post-operation, such as 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 from Mar 2023 to Jun 2023.

Vasectomy Procedure

Scrotal coats of all animals were clipped, shaved, and cleansed with soap and antiseptic solution was applied, which constituted 70% Alcohol. This is followed by sedation by intramuscular injection of Xylazine 2% (Alfasan, Woerden, Holland) with a dose of (0.2 mg/kg B.W) and lidocaine 2% (Rotexmedica, Trittau, Germany) as a local infiltration anaesthesia subcutaneaous by a dose of 4 mg/Kg B.W. 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) coated at a distance of 1 cm, with an excision removing ½ cm between these ligatures. This is followed by returning ligature ends. The incised skin was sutured by a simple interrupted suture pattern with non-absorbable synthetic suture material

(#1) monofilament polyamide. Another ductus difference was made in a similar manner, which depends on [5].

Sexual Assessment

Buck ejaculate was collected using artificial vagina into a warmed glass tube at 37ºC prior to vasectomy operation 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 by a graduated glass tube in millilitres. Sperm motility was evaluated considering progressive sperm movement, a visual score (0-100%), on the warmed slide (37ºC) under a electronic microscope (×400) [6]. These investigations were performed on all the Bucks before the operation as a normal (control) grade, which symbolised the (0) period, also at 4, 7, 10, 14, and 21 days post-operation for each male.

Testosterone Assessment

Five millilitres of blood were collected using a disposable syringe from the jugular vein of bucks in the morning hours. Samples were placed in sterile transport tubes to isolate serum, and kept at -20 °C until determination of testosterone levels by 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]. These samples were collected at (0) period as a normal grade (control) prior to operation, then at (1, 2, 3, and 4) months post-operation.

Histopathological Assessment

Testicular biopsies of 1cm3 were obtained for microscopic examination at 1, 2, 3 and 4 months post-operation after castration for 1, 2, 3 and 4 groups respectively to study pathological changes. Biopsies were fixed in 10% neutral buffered formalin for 72 hours, dehydrated, cleared in xylene and embedded in paraffin wax using routine methods. The sections were cut to a thickness of 5-6µm de-waxed, cleared in xylene, rehydrated and stained using hematoxylin

and eosin to examine under the light microscope for total assessment. Leydig cells were counted depending on the method [8], the number of Leydig cells among the three seminiferous tubules. A total of twenty 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

The Statistical Package for the Social Sciences (SPSS) version 25 software was used to effect study factors in traits. 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.

Author 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.

Acknowledgments

The authors thankful to Faculty of Veterinary Medicine of Kufa University for providing the facilities. The authors did not receive any funds for this study.

Conflicts of interest

The authors declare there is no conflict of interest.

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Figure legends

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 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 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 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).

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

Table 2. The effects of testosterone levels at prior and post vasectomy operations.

Table 3. The leydig cells count at post vasectomy operations.