

INVESTIGATION OF SURGICAL VASECTOMY EFFECTS ON SEMINAL AND HORMONAL PROFILES IN MALE RABBITS

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Abstract

Background: Vasectomy is a common surgical method used for male sterilization by cutting or blocking the vas deferens. Studying its effects in rabbits helps understand how this procedure influences semen quality and hormone levels. This research provides useful information about reproductive physiology and potential impacts on male fertility.

Objective: To evaluate how surgical vasectomy affects the semen quality, gonadal measurements, and hormone levels in male rabbits to understand its impact on reproductive functions.

Methodology: Six pairs (n=12) of mature adult male rabbits weighing 1.42 ± 0.09 kg were recruited for the experiment. Animals were divided into three experimental groups, and each group consisted of four rabbits. One control group (sham operated), second unilateral vasectomy group and third bilateral vasectomy group. Experimental vasectomy was done carefully unilateral in second group and bilateral in third group. Semen and blood samples were collected and assayed for sperm motility, sperm morphology, semen fructose level, semen pH and testosterone at the 15 days of vasectomy operation with the interval of each 15 day for 12 weeks.

Results: Sperm motility, sperm tail, seminal pH level and testosterone level in serum of unilateral vasectomized and bilateral vasectomized group declined as compared to control group. Statistically, there was significantly lower

concentration of testosterone in bilateral vasectomized group than unilateral vasectomized group and control group.

Conclusion: sperm motility, sperm tail, seminal pH level and testosterone level were high and significantly decreased in bilateral vasectomized rabbits' group.

INTRODUCTION

Vasectomy is now widely accepted as a safe and dependable method of contraception for couples (Schwingl and Guess, 2000; Cox et al., 2002). Vasectomy is used by more than 40 million couples worldwide to terminate pregnancy. This process is quick and trusty with low rates long-term complication, i.e., in the rank 0.08–0.10%. Vasectomy is a surgical procedure to cut and tied vasa deferentia for male neutering to stop fertilization of a female through sexual intercourse. Vasectomy is a method used successfully worldwide for male contraception and in 2015 in the United States approximately 527,476 vasectomies were utilized. An evolution of trend for use of vasectomy revealed that 82% of vasectomies were performed during 2014 and 2015 in USA (Ostrowski et al., 2018). In the United States compared to female contraception, male neutering (vasectomy) is the most efficacious, with lesser ratio of complications low cost-effective and only long-acting form of contraception. Along these advantages, in the United States of America, vasectomy is performed at low than half the ratio of female neuterring. In addition, it is utilized at less numbers among the populations of Latino and Black, as compared to female neutering (Shihi et al., 2011). The unusual growth of population is recognized as an alarming situation to the international community. It is high obstructions for socioeconomic growth. Vasectomy is one of the techniques to control the population by offering reliable procedure of contraception and which has been approved by the World Health Organization (Hosseini and Abdi, 2012) in different countries and religions there are religious and ethical challenges beside different issues for family planning, contraception, abortion, female sterilization and male vasectomy. Presently, all over the world to prevent pregnancy about more than 40 million twosomes rely on it (Vrijhof et al., 2004).

The vas deference is a structure of tube shape comprises of a mucosal inner layer and three muscular layers that carry sperm to the ejaculatory duct from the cauda epididymis. In this surgical procedure luminal continuity of the vas deferens is interrupted and thus interferes with transportation of spermatozoa from the testis and epididymis into the urethra. Injury to the vas deference during vasectomy may affect negatively on male fertility resulting from enlarged diameter of seminiferous tubular, sperm maturing process and obstruction of spermatogenesis (Singh and Charvarty, 2000; Duru et al., 2013). The vas deferens unilateral ligation results in a bilateral flaw in spermatogenesis and in the epididymal sperm maturation procedure (Ikeda and Nikolaos, 2000). In their study observed post vasectomy motility, declines in sperm count and other morphological aberrations seen in sperm morphology (Kim et al., 2001). The side effects such as, painful nodules, sperm granuloma and the stasis of epididymis (Huang et al., 2010).

Vasectomy experiments on testis of rat, guinea pig, and monkey revealed that histo-archetectural changes in testis occurs after vasectomy. Vasectomy in rat, guinea pig, rabbit, dog and monkey has shown regression of seminiferous tubules. Singh and Charvarty (2000) observed the presence of macrophages in the lumen of the rete testis and seminiferous tubes of the vasectomized rat that reflected the elimination of degenerated spermatozoa. In the seminiferous tubules of Vasectomized mice showed degenerative changes; these changes include tubules vacuolations, basement membrane thickening and sloughing of immature germ cells in the tubules. Lumens of several seminiferous tubules had sloughed spermatogenic cells and enormous quantities of spermatids. Highly congested blood vessels were present in the interstitial tissue of few sections and big necrotic parts covering certain of the tubules together with the eosinophilic material (edema) of

intertubular and with the scant mononuclear cell's infiltration. Properly not developed germ cells were seen in disarray of few tubules. Various tubules were near entirely damaged (with spermatogenic cells vacuoles) leaving outsized areas of interstitial space edema and few testes showed numerous interstitial fibrosis with the tubules vacuolations and thickening of the basement membrane (Salman *et al.*, 2012). The histo-archetectural damages result in impaired function of testis that appears as significant decrease in testosterone in vasectomized rat and also declined sperm count and motility with alteration in sperm morphology (Fio *et al.*, 2013). Testosterone serum level in the vasectomized animal's response to bilateral testicular weight and human chorionic gonadotropin and were significantly lower (Ikeda and Nikolaos, 2000).

MATERIALS AND METHODS

Animals and study site

Twelve apparently clinically and healthy, adult rabbits about 408 ± 14.7 days of age, weighing 1.42 ± 0.09 kg body weight were purchased from open market of Hyderabad and kept at animal house of Faculty of Animal Husbandry, SAU, Tandojam.

Experimental design

After an adaptation period of two weeks all rabbits were grouped as control (group-1), unilateral vasectomy (group-2) and bilateral vasectomy (group-3). Then the rabbits were tagged according to their weight and divided into three groups Group-1 (Control) $n=4$, [blue color tag=1, 2, 3, 4], Group-2 (Unilateral Vasectomy) $n=4$ [green color tag=5, 6, 7, 8]. Group-3 (Bilateral Vasectomy) $n=4$ [white color tag= 9, 10, 11, 12]. Rabbits of all groups were kept under observation in different cages. Vasectomy operations were performed in hygienic environment, anesthesia to each rabbit of unilateral vasectomized group-2 and bilateral vasectomized group-3 were induced by intramuscular injection of ketamine and xylazine at a dose 35 mg/kg and 5 mg/kg respectively. In the vasectomy a scrotal incision was made to expose the vas deferens, about 2 to 3 cm length of it were cut out with the two ends ligated. After

operations, each rabbit was treated with 80,000-unit penicillin with intramuscular injection for three days and kept the wounds dry with pyodine to avoid infection. The rabbits were raised as routine in the animal house center. Data collection started on the 15th day after operation with an interval of 15 days for 12 weeks.

Semen analysis

Fresh semen drop was put on slide examine under microscope using 40x for motile and non-motile sperm ratio pre and post vasectomy Semen were preserved semen samples were used for sperm morphology determined by staining with eosin - nigrosin stain under microscope using 40x, pH was determined with pH strip and fructose was determined with glucometer using fresh seminal fluid.

Measurement of testosterone

Blood 4-5 ml collected from jugular vein using 22 gauge needle and from ear vein using 22 gauge butterfly cannula then putted in centrifugation machine for 25 minutes 5000rpm for serum collection. Testosterone level was measured in serum samples obtained from each rabbit of control group, unilateral vasectomized group and bilateral vasectomized group by using Enzyme Linked Immuno Assay Kit.

Correlation between studied parameters

Obtained for testosterone level, sperm motility, fructose level and seminal fluid pH in all groups will be analyzed to evaluate correlation through statical software STATIX-10 and will be described as positive or negative correlation

STATISTICAL ANALYSIS

Data obtained were analyzed using software STATIX-10 by ONE-WAY ANOVA with Turkey's test to compare between the groups and results expressed as mean + SD. $P < 0.05$ was considered significant.

RESULTS

Sperm motility

Mean of Sperm motility was $65.250 \pm 0.9167\%$ in group-1, $42.750 \pm 5.8523\%$ in group-2 and

26.000±3.915% group-3. Statistical analysis showed that there was higher ($P<0.01$) sperm motility in group-1 than group-2 and group-3,

further analysis showed high sperm motility ($P<0.05$) in group-2 than group-3 (Table 1).

Table: 1 Sperm motility in group-1, group-2 and group-3

Variable	N	Mean	SD
Group1	4	65.250 ^a	±0.9167
Group2	4	42.750 ^b	±5.8523
Group3	4	26.000 ^c	±3.915

$P\leq 0.05$

LSD 8.0991

Sperm morphology

Head of sperm mean was 60.00±0.82 mm in group-1, 46.25±8.54 mm in group-2 and 38.50±9.95 mm in group-3. Sperm body was 44.75±7.68 mm in group-1, 36.25±2.50 mm in group-2 and 26.25±6.29 mm in group-3. Tail of sperm was 36.75±5.38 mm in group-1, 27.50±3.32 mm in group-2 and 12.00±2.16 mm in group-3. Statistically, there was significantly higher ($P<0.05$) head of sperm in group-1 as

compare to group-2 and group-3 but there was no significant difference between group-2 and group-3. Sperm body was significantly larger ($P<0.05$) in group-1 than group-2 and group-3 but there was no significant difference in group-2 and group-3. Sperm tail was significantly shorter ($P<0.05$) in group-3 as compared to group-1 and group-2, but there was no significant difference in between the sperm tail of group-1 and group-2 (Table 2).

Table: 2 Sperm morphology in rabbits' group-1, group-2 and group-3

Variable	N			Mean			SD		
	Head	Body	Tail	Head	Body	Tail	Head	Body	Tail
Group1	4	4	4	60.000 ^a	44.750 ^b	36.750 ^{bc}	±0.8165	±7.6757	±5.3774
Group2	4	4	4	46.250 ^{ab}	36.250 ^{bc}	27.500 ^c	±8.5391	±2.5000	±3.3166
Group3	4	4	4	38.500 ^{bc}	26.250 ^c	12.000 ^d	±9.9499	±6.2915	±2.1602

$P\leq 0.05$

LSD 14.228

Seminal fluid fructose level

Seminal fluid fructose level mean was 756.50±10.599 µg/ml in group-1, 769.00±5.03 µg/ml in group-2 and 779.00±0.82 µg/ml in

group-3. Statistically, there was significantly lower ($P<0.05$) seminal fluid in group-1 than group-2 and group-3, but statistically, there was no significant difference in group-1 and group-2 (Table 3).

Table: 3 Seminal fluid fructose level in rabbits' group-1, group-2 and group-3

Variable	N	Mean µg/ml	SD
Group1	4	756.50 ^b	±10.599
Group2	4	769.00 ^{ab}	±5.0332
Group3	4	779.00 ^a	±0.8165

P≤0.05

LSD 13.405

Seminal fluid pH value

Seminal fluid pH value mean was 7.55±0.13 in group-1, 6.80±0.245 in group-2 and 6.40±0.35 in group-3. Statistically, pH of group-1 was

significantly higher (P<0.05) than group-2 and group-3 but there was no significant difference in group-2 and group-3 (Table 4).

Table: 4 Seminal fluid pH value in rabbits' group-1, group-2, and group-3

Variable	N	Mean	SD
Group1	4	7.5500 ^a	±0.1291
Group2	4	6.8000 ^b	±0.2449
Group3	4	6.4000 ^b	±0.3464

P≤0.05

LSD 0.5054

Testosterone level

Testosterone level mean was 3.93±1.78 ng/ml in group-1, 2.84±1.36 ng/ml in group-2 and

0.05±0.03 ng/ml in group-3. Statistically, there was significantly lower concentration of testosterone in group-3 than group-2 and group-1 (Table 5).

Table: 5 Testosterone level in rabbits' group-1, group-2, and group-3

Variable	N	Mean ng/ml	SD
Group1	4	3.9250 ^a	1.7821
Group2	4	2.8350 ^a	1.3651
Group3	4	0.0450 ^b	0.0332

P= 0.6475

LSD2.5587

Correlation between testosterone level, sperm motility, fructose level and seminal fluid pH

Results depicted in table-6 show that there is positive correlation between testosterone level

with sperm motility and seminal fluid pH, whereas fructose level was negatively correlated with testosterone level and sperm motility was also negatively correlated with pH level.

Table: 6 Correlation of testosterone level, sperm motility, fructose level, and seminal fluid pH value in group-1, group-2 and group-3

Variable	Testosterone level	Sperm motility	Fructose level	Seminal pH
TL	1	1	-1	1
SM	1	1	1	1
FL	-1	-1	1	-1
S-Ph	1	1	-1	1

DISCUSSION

From the current study it was concluded that in long term vasectomized New Zealand Big Ears White Rabbits the histological changes were observed under the light microscopy in the testis, epididymis and vas deferens, seminiferous epithelium became thinned and loose, cells were in disarray position. The seminiferous tubules were expanded. Spermatids and spermatozoa could not be seen clearly in the seminiferous tubules the epididymis was expressively increased and large in the vasectomy group. The pseudo stratified columnar epithelial cells were deformed and congested. There was enough debris accumulated in the epididymis lumen. The epithelium was deformed and compressed and at the basement, fibroplasias could be seen when the vas deferens were ligated for one year the cells were severed more apoptosis. Approximately all the cell's DNA was broken, indicated brown stained. The apoptosis index was 96% in the vasectomy group (Wang *et al.*, 2012). This apoptosis may be due to auto-immune attack. Auto-immune pathology in humans above 50% effect post-vasectomy. The immunological alterations, if any, following vasectomy can be revealed in 2 patters: those that anatomy, either microscopic, or gross and adversative effect in the reproductive tract physiology could be due to the existence of antibody. Gross anatomical changes consist of spermatid granulation development and cystic swelling at the spot of surgery. Included within the tissue-level alteration in experimental auto-immune orchitis or post-vasectomy are unstable position of vacuolization of the sertoli cells, premature exfoliation of immature germ cells, spermatid nucleus and acrosome falsification, a late spermatids retaining and deterioration in the epithelium, in some pathological situations such ligation of vas-deferens, due to phagocytosis of the spermatozoa the soluble antigens of the sperm penetrate into the blood circulation and autoantibodies generation occurred . In sera sperm agglutinating antibodies have been observed on third or fourth day of post vasectomy, but they appear among 6 weeks to 6 months in the sera. The non-vasectomized individuals circulating immobilizing sperm antibodies are detected 1%-

3%. After operation approximately 31% in the period of sex months a person develops these antibodies, in non-vasectomized male less than 2% swollen sperm head antibodies and in vasectomized males within a year 28-33% are found. In experimental animals' existence and post-vasectomy circulating immune complications effects are reviewed with reference to reported increased occurrence of auto-immune orchitis and atherosclerosis (Shanhani and Hattikudur, 1981; Goldacre *et al.*, 2007). Significant reduction in sperm motility in unilateral and bilateral vasectomized rabbits was observed in present study, which agrees with findings of Amini *et al.*, (2013) on sperm parameters in adult male Blab/c mice show that vasectomy in mice leads to early failure of spermatogenesis and reduced sperm motility. This effect of vasectomy has been reported in other studies as well. For example, Ren *et al.*, (2011), who investigated the effect of short period vasectomy on sperm motility in adult male rats, found that vasectomy can harm spermatogenesis, reducing sperm motility starting from day 60 after vasectomy. Silber *et al.*, (1995) have suggested that in cases of chronically obstructed epididymis, sperm suffer changes related to old age with a significant decrease in motility towards the distal part. Fio *et al.*, (2013) also reported same that in the study of unilaterally vasectomized mature male African giant rat (*Cricetomys gambianus*) histo-archetectural of testis damaged so resulted in impaired function of testis that appears as significant declined in motility. Post vasectomy Seppan and Kirishnaswamy (2014), reported sperm granuloma formation in the juxta-epididymal segment of the occluded vas deferens became very short for deposition of spermatozoa with surgical procedure of vasectomy so the autoimmune attack occur with accumulation of immunoglobulin (IgG) deposition as well as granulomatous reaction in the epididymis and vas deferens which effect the sperm quality and Pelfrey *et al.*, (1982) reported that in the study of vasectomy reversal in 29 men with the presence of infertility stated that in 65% of the total specimens sperm show only 10% normal head with 2-64% coiled or shortened with abnormal tail due alteration epididymal

physiology. So, the present study shows the same result that only head of sperm was normal in unilateral and bilateral the sperm body was observed same in unilateral and control group, but it was significantly unseen in bilateral. Sperm tail was totally unseen in unilateral and bilateral.

The seminal fluid pH value was studied by Caflisch and Dubose (1990) in Sprague-Dawley adult male rats in unilateral vasectomy concluded that at the thirty day of the post-vasectomy resulted the significantly drop in the pH of the epididymal duct and testis seminiferous tubules of the rat may due to the decreased exchange of the vascular carbon dioxide (CO₂) in the plexus of pampiniform and direct decreasing the transportation of bicarbonate [HCO₃⁻] in to the seminiferous tubules. Present study resulted the same as stated.as compared to control group in unilateral and bilateral vasectomized rabbits show high significant acid development in the seminal fluid.

Fructose level in seminal fluid was observed increased in unilateral and bilateral in present study. That was high significantly evaluated in both vasectomized groups as compared to the control group of the rabbits resulted in accordance with those of Trang *et al.*, (2018) reported seminal fructose concentration in man infertility resulted that fructose is the source of energy for sperm in seminal fluid due to vasectomy sperm motility become slow are even restricted and no consumption of fructose so the fructose concentration increased in the seminal fluid.

In unilateral vasectomy of the male AGR (African giant rat) they described that impairment in the ipsilateral testicular role in the testis due to the obstruction in the vas deferens developed the large pressure in the seminiferous tubules and epididymis leads by the no withdrawal of spermatozoa from the seminiferous tubules and epididymis (Fio *et al.*,2013). This may result the fibrosis in the interstitial fibrosis of the testes and epididymal obstruction which effect the testosterone.

CONCLUSION

It was concluded from the results of the present study that vasectomy significantly influence on

levels of fructose level that increased in seminal fluid of vasectomized rabbits. In semen. Whereas gonadal hormone level, sperm motility and seminal fluid pH decreased significantly in both unilateral and bilateral vasectomized rabbits. Morphological changes were also apparent in sperms of vasectomized rabbits as reduced tail size, body and head of sperm. Vasectomy resulted in decreased gonadal hormone level, pH of semen and increased in fructose levels.

Author's contribution

Conceptualization: IU, ABK

Methodology: SAS, AH

Formal analysis: JK, MI, SA

Writing review and editing: SN, TA, MU

All authors have read and agreed to the published version

Conflict of Interest

All authors declare no conflict of interest

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