



RESEARCH ARTICLE

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### Hematology of Langur Monkeys following Short and Long Term of Vasectomy

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#### **ABSTRACT**

Vasectomy is considered as one of the safest, most effective, one time method of male contraception and being practiced worldwide. Since the method is taken up as permanent mode of contraception due to its reversibility problem, the immediate concern associated with it is, its side effects leading to any disease. Over the years vasectomy has been suspected of links with many health conditions. In this view the present investigation was focused on the hematological studies following short (six months) and long term (24 months) of vasectomy indicating the health of the subject following both durations of vasectomy. Unaltered hematological and physiological measures demonstrated no disease state or adverse effect of vasectomy on the general health of the animal subjects studied.

**Key words:** Hematology, Langur Monkeys, Vasectomy

#### **INTRODUCTION:**

Since Vasectomy is taken up by the couples who have completed their family and do not look for reversibility, concern arises of its side effects. It has been reported to be associated with risk of heart diseases, kidney, arthritis, multiple sclerosis, gout and other immune diseases. Even musculoskeletal disorders, impotence, depression and cancers of testicles and prostate. Therefore the present study plans to look into its this aspect through blood and serum studies which are considered to be the indicators of these diseases.

#### **OBJECTIVE:**

The aim of the study was to investigate the blood and serum parameters from six and twenty four months of vasectomized langur monkeys to measure its variations, if any, to find out the health status of the animals.

#### **ANIMAL MODEL:**

Adult male langurs (*Presbytis entellus entellus* Dufresne) were used in the study.

#### **EXPERIMENTAL DESIGN:**

##### **Intact Control:**

Unoperated three animals served as control.

##### **Sham Operations:**

Six animals were sham operated. It involved immobilization of vas without cutting and ligating the vas.

**Vasectomy:**

Bilateral vasectomy was performed in 6 six adult langurs monkeys for six month and twenty four months under sterile conditions. Animals were anaesthetized with intravenous injection of intraval sodium (20 mg/kg/b.wt) through saphenous vein puncture with the help of hypodermic syringe. Vas deferens of both side were exposed and a piece of 0.5–1 cm was removed from each side.

**Blood Evaluation:**

Blood samples were collected from conscious monkeys by saphenous vein puncture using heparinized hypodermic syringe (Needle No. 21) prior to and following 0.5, 1, 2, 3, 6, 9,12,15,18 and 24 moths of vasectomy. Haematological parameters RBC, total WBC count were made by routine standard clinical methods by an improved Neuberer's haemocytometer (Lynch et al., 1969) hemoglobin concentration was measured by Cynmethemoglobin method using Fisher's hemophotometer, model 55 (Crosby et al., 1954), packed cell volume (PCV), (Natelson, 1951) and haematological indices MCV, MCH, MCHC and MCAT were also calculated.

**Biochemical Investigation:**

Blood samples collected were allowed to clot at room temperature for 15 to 20 minutes. Clotted samples were than centrifuged at 3000RPM for 10 minutes. Serum was separated and stored at -20°C in small aliquots.

Blood urea (Archer and Robb, 1967), total cholesterol (Zlatkis et al., 1953), alkaline phophatase (Fiske and Subbaraw, 1955), lactic dehydrogenase (Cabaud and Wroblewski, 1958), bilirubin (King and Coxon, 1950), Transaminases : SGOT and SGPT (Reitman and Frankel, 1957) and Serum electrolyte; Na<sup>+</sup> and K<sup>+</sup> by flame photometry (John, 1960) were estimated.

**RESULTS:**

The basic haematological parameters (Haemoglobin, RBC, WBC, and PCV) did not show any alteration in sham operated and vasectomized animals when compared with the values of intact animals for the same parameters.

Standard haematological indices (CI, MCH, MCHC, MCV and MCAT) were also found in the same range in all the groups of animals i.e, intact, sham operated and vasectomized animals. Serum total cholesterol, bilirubin, LDH, SGOT, SGPT, alkaline phosphatase, sodium and potassium and blood urea was found in comparable range in all the groups of animals.

**Intact Animals:**

The mean values of basic haematological parameters i.e. Hemoglobin (13.75±0.40 gm/100ml), RBC (6.10±0.18 million/mm<sup>3</sup>), WBC (11.77±0.66.10<sup>3</sup>mm<sup>3</sup>) and PCV (45.17±2.04%) were within control range.

Similarly the mean value of haematological indices i.e color index (CI) (0.93±0.03) mean corpuscular volume (MCV) (69.39±1.48µ<sup>3</sup>) mean corpuscular haemoglobin (MCH) (19.21±0.65pg) mean corpuscular haemoglobin concentration(MCHC) (23.45±0.02%) and mean corpuscular average thickness (MCAT) (2.13±0.09µ) were also found within control range.

**Blood / Serum Biochemistry:**

Blood urea (25.14±0.58 mg/100ml),alkaline phosphatase (ALP) (6.31±0.38 BU/100ml), serum glutamic oxalacetic transaminases (SGOT) (16.97±0.57FU/ml),

serum glutamic pyruvic transaminases (SGPT) ( $18.44 \pm 0.61$  FU/ml), lactic dehydrogenase (LDH) ( $436.22 \pm 33.37$  IU/ml), bilirubin ( $0.61 \pm 0.02$  mg/100 ml), total cholesterol ( $206.33 \pm 7.18$  mg/100ml), sodium ( $52.11 \pm 2.49$  mEq/l) and potassium ( $5.69 \pm 0.17$  mEq/l) were all within control range.

### Sham Operated and Vasectomized Animals:

The mean value of all the haematological parameters and hematological indices various parameters studied for blood/serum biochemistry of both the groups of animals i.e sham operated and vasectomized were comparable with those of intact animals. (Table 1 & 2).

**Table 1:** Haematological Measures Prior to and Following Vasectomy in Langur Monkey

Duration	RBC ( $\times 10^6$ /Cmm)		WBC ( $\times 10^6$ /Cmm)		Haemoglobin (gm/100ml)		Haematocrit (%)	
	Sham Operated Control	Vasectomy	Sham Operated Control	Vasectomy	Sham Operated Control	Vasectomy	Sham Operated Control	Vasectomy
Pre-Vasectomy	$6.10 \pm 0.18$	$5.85 \pm 0.24$	$11.77 \pm 0.66$	$12.12 \pm 0.53$	$13.75 \pm 0.40$	$13.42 \pm 0.38$	$45.17 \pm 2.04$	$44.89 \pm 1.49$
Vasectomy 0.5 Months	$5.87 \pm 0.22$	$5.79 \pm 0.32$	$12.67 \pm 0.42$	$13.01 \pm 0.46$	$13.94 \pm 0.36$	$13.61 \pm 0.39$	$44.93 \pm 2.04$	$45.32 \pm 2.01$
1 Month	$6.24 \pm 0.41$	$6.24 \pm 0.22$	$12.73 \pm 0.51$	$12.33 \pm 0.71$	$13.17 \pm 0.35$	$13.08 \pm 0.65$	$46.76 \pm 2.74$	$44.63 \pm 2.62$
2 Months	$6.10 \pm 0.19$	$6.40 \pm 0.25$	$11.70 \pm 0.66$	$12.15 \pm 0.67$	$13.81 \pm 0.41$	$14.20 \pm 0.31$	$47.09 \pm 2.35$	$46.58 \pm 2.94$
3 Months	$6.25 \pm 0.31$	$6.35 \pm 0.23$	$11.90 \pm 0.52$	$11.41 \pm 0.51$	$13.94 \pm 0.21$	$14.03 \pm 0.24$	$46.24 \pm 1.90$	$44.63 \pm 1.90$
6 Months	$5.97 \pm 0.13$	$6.12 \pm 0.17$	$11.64 \pm 0.33$	$11.51 \pm 0.36$	$13.89 \pm 0.29$	$13.86 \pm 0.27$	$45.64 \pm 1.43$	$44.62 \pm 2.09$
9 Months	$6.07 \pm 0.24$	$6.18 \pm 0.22$	$11.47 \pm 0.45$	$10.89 \pm 0.96$	$13.95 \pm 0.30$	$14.00 \pm 0.36$	$45.68 \pm 2.34$	$48.80 \pm 1.88$
12 Months	$6.00 \pm 0.41$	$6.09 \pm 0.29$	$12.22 \pm 0.75$	$12.57 \pm 1.00$	$13.87 \pm 0.36$	$13.55 \pm 0.41$	$46.88 \pm 2.62$	$47.89 \pm 2.10$
15 Months	$6.03 \pm 0.12$	$6.21 \pm 0.09$	$11.50 \pm 0.73$	$11.42 \pm 0.39$	$14.60 \pm 0.26$	$14.25 \pm 0.26$	$46.83 \pm 3.13$	$49.20 \pm 2.06$
18 Months	$6.17 \pm 0.19$	$6.19 \pm 0.22$	$11.52 \pm 0.45$	$11.78 \pm 0.39$	$14.32 \pm 0.26$	$14.00 \pm 0.58$	$42.60 \pm 1.45$	$45.20 \pm 1.54$
21 Months	$6.17 \pm 0.25$	$6.30 \pm 0.23$	$12.55 \pm 0.53$	$12.28 \pm 0.40$	$14.17 \pm 0.30$	$13.80 \pm 0.64$	$44.82 \pm 1.63$	$45.90 \pm 2.15$
24 Months	$6.10 \pm 0.30$	$6.59 \pm 0.15$	$11.90 \pm 0.38$	$12.02 \pm 0.33$	$14.20 \pm 0.24$	$13.67 \pm 0.28$	$45.07 \pm 1.77$	$47.55 \pm 0.41$

**Table 2:** Physiological Measures Prior to and Following Vasectomy in Langur Monkey

Duration	Blood Urea (mg/100ml)		ALP (BU/100ml)		SGOT (FU/ml)		SGPT (FU/ml)		LDH (IU/ml)		Bilirubin (mg/100ml)		Cholesterol (mg/100ml)		Na <sup>+</sup> (mEq/l)		K <sup>+</sup> (mEq/l)	
	Sham operated control	Vasectomy	Sham operated control	Vasectomy	Sham operated control	Vasectomy	Sham operated control	Vasectomy	Sham operated control	Vasectomy	Sham operated control	Vasectomy	Sham operated control	Vasectomy	Sham operated control	Vasectomy	Sham operated control	Vasectomy
Pre-Vasectomy	$26.14 \pm 0.58$	$25.50 \pm 1.36$	$6.31 \pm 0.38$	$6.22 \pm 0.25$	$16.97 \pm 0.57$	$16.08 \pm 0.43$	$18.44 \pm 0.61$	$18.48 \pm 0.67$	$436.22 \pm 33.37$	$427.25 \pm 31.20$	$0.61 \pm 0.02$	$0.67 \pm 0.04$	$206.33 \pm 7.18$	$207.33 \pm 6.09$	$152.11 \pm 2.49$	$152.33 \pm 2.37$	$5.69 \pm 0.17$	$5.55 \pm 0.17$
Vasectomy 0.5 Months	$24.60 \pm 0.87$	$25.69 \pm 0.83$	$5.99 \pm 0.23$	$6.29 \pm 0.17$	$15.77 \pm 0.51$	$16.29 \pm 0.77$	$17.87 \pm 0.77$	$17.93 \pm 0.81$	$356.56 \pm 25.03$	$391.00 \pm 33.42$	$0.56 \pm 0.03$	$0.61 \pm 0.04$	$203.78 \pm 3.57$	$202.67 \pm 19.98$	$152.44 \pm 2.56$	$153.22 \pm 2.29$	$5.70 \pm 0.19$	$5.71 \pm 0.18$
1 Month	$26.94 \pm 0.72$	$26.32 \pm 0.82$	$6.05 \pm 0.18$	$5.96 \pm 0.20$	$16.99 \pm 0.50$	$16.53 \pm 0.45$	$19.28 \pm 0.63$	$18.57 \pm 0.75$	$413.44 \pm 33.87$	$409.67 \pm 41.10$	$0.65 \pm 0.04$	$0.68 \pm 0.06$	$216.56 \pm 7.09$	$222.56 \pm 6.78$	$156.44 \pm 2.83$	$155.78 \pm 2.03$	$5.62 \pm 0.19$	$5.50 \pm 0.23$
2 Months	$28.46 \pm 1.05$	$29.22 \pm 1.03$	$5.98 \pm 0.21$	$6.03 \pm 0.33$	$16.78 \pm 0.52$	$17.02 \pm 0.57$	$18.79 \pm 0.89$	$18.51 \pm 0.81$	$457.56 \pm 42.36$	$483.67 \pm 51.57$	$0.62 \pm 0.03$	$0.74 \pm 0.06$	$204.44 \pm 6.09$	$211.89 \pm 7.26$	$155.56 \pm 3.65$	$155.11 \pm 3.98$	$5.82 \pm 0.18$	$5.47 \pm 0.16$
3 Months	$24.98 \pm 1.02$	$25.73 \pm 0.83$	$6.26 \pm 0.33$	$6.26 \pm 0.34$	$17.30 \pm 0.63$	$16.86 \pm 0.52$	$18.48 \pm 0.71$	$18.54 \pm 0.87$	$423.56 \pm 46.17$	$467.67 \pm 33.46$	$0.62 \pm 0.02$	$0.67 \pm 0.03$	$205.00 \pm 6.29$	$210.44 \pm 3.34$	$154.56 \pm 3.49$	$155.22 \pm 4.56$	$5.58 \pm 0.18$	$5.68 \pm 0.18$
6 Months	$26.48 \pm 1.19$	$25.01 \pm 1.61$	$5.99 \pm 0.38$	$6.17 \pm 0.28$	$16.80 \pm 0.59$	$16.72 \pm 0.54$	$18.45 \pm 0.69$	$18.93 \pm 0.76$	$404.00 \pm 33.16$	$440.00 \pm 25.03$	$0.65 \pm 0.03$	$0.64 \pm 0.05$	$203.89 \pm 5.99$	$205.67 \pm 5.67$	$155.56 \pm 3.73$	$152.22 \pm 1.88$	$5.49 \pm 0.27$	$5.49 \pm 0.17$
9 Months	$23.77 \pm 1.59$	$22.60 \pm 1.43$	$6.12 \pm 0.19$	$6.19 \pm 0.21$	$16.63 \pm 0.49$	$16.04 \pm 0.15$	$18.68 \pm 0.97$	$17.93 \pm 1.00$	$405.83 \pm 25.89$	$414.17 \pm 16.76$	$0.65 \pm 0.03$	$0.69 \pm 0.05$	$206.50 \pm 9.30$	$207.67 \pm 5.14$	$153.33 \pm 6.48$	$154.00 \pm 2.44$	$5.65 \pm 0.26$	$5.44 \pm 0.16$
12 Months	$25.88 \pm 1.14$	$25.75 \pm 0.86$	$6.19 \pm 0.44$	$6.09 \pm 0.38$	$16.55 \pm 0.88$	$15.85 \pm 0.44$	$18.81 \pm 0.91$	$17.24 \pm 0.75$	$394.83 \pm 25.67$	$391.33 \pm 7.64$	$0.70 \pm 0.05$	$0.72 \pm 0.05$	$208.33 \pm 8.82$	$218.50 \pm 5.10$	$156.17 \pm 4.24$	$155.33 \pm 2.85$	$5.45 \pm 0.20$	$5.49 \pm 0.14$
15 Months	$23.47 \pm 1.16$	$23.05 \pm 0.90$	$5.80 \pm 0.31$	$6.02 \pm 0.37$	$17.11 \pm 0.85$	$17.53 \pm 0.88$	$18.37 \pm 0.83$	$19.10 \pm 0.90$	$406.50 \pm 22.54$	$411.33 \pm 37.97$	$0.58 \pm 0.03$	$0.57 \pm 0.03$	$204.17 \pm 5.39$	$200.50 \pm 7.74$	$152.50 \pm 2.35$	$154.00 \pm 3.02$	$5.85 \pm 0.25$	$5.88 \pm 0.16$
18 Months	$25.02 \pm 1.33$	$24.30 \pm 1.21$	$5.62 \pm 0.22$	$5.70 \pm 0.33$	$16.87 \pm 0.80$	$17.13 \pm 0.81$	$18.87 \pm 1.30$	$18.85 \pm 0.95$	$406.00 \pm 39.62$	$417.83 \pm 52.43$	$0.59 \pm 0.03$	$0.57 \pm 0.03$	$201.67 \pm 11.08$	$201.67 \pm 14.70$	$148.83 \pm 4.59$	$153.83 \pm 3.98$	$5.92 \pm 0.18$	$6.04 \pm 0.25$
21 Months	$25.03 \pm 2.19$	$25.57 \pm 1.70$	$5.85 \pm 0.35$	$6.16 \pm 0.39$	$16.81 \pm 0.82$	$16.94 \pm 0.78$	$18.55 \pm 0.84$	$19.82 \pm 0.54$	$411.00 \pm 45.02$	$433.67 \pm 39.85$	$0.59 \pm 0.03$	$0.59 \pm 0.05$	$206.67 \pm 8.82$	$208.33 \pm 10.14$	$153.33 \pm 4.01$	$151.17 \pm 6.05$	$5.72 \pm 0.27$	$5.59 \pm 0.24$
24 Months	$25.52 \pm 0.89$	$24.40 \pm 1.19$	$5.83 \pm 0.45$	$5.70 \pm 0.31$	$16.34 \pm 0.55$	$17.08 \pm 0.79$	$18.16 \pm 0.91$	$19.12 \pm 0.79$	$348.67 \pm 19.22$	$390.67 \pm 40.83$	$0.58 \pm 0.04$	$0.59 \pm 0.03$	$199.83 \pm 7.03$	$200.00 \pm 8.16$	$155.00 \pm 3.18$	$154.67 \pm 4.81$	$5.27 \pm 0.18$	$5.52 \pm 0.23$

**DISCUSSION:**

Over the years vasectomy has been suspected of links with many health conditions including an increased risk of heart disease, reduced or increased lipid levels, kidney diseases, arthritis, multiple sclerosis gout and other autoimmune diseases, musculoskeletal disorders, impotence, depression and recently cancer of the testicles and prostate. In a large group study involving almost 22,000 men, vasectomized men had similar or lower rate of 98 diseases, including various cancers, autoimmune diseases heart disease than control who did not have vasectomies (Massey et al., 1984). No alteration in hemoglobin, erythrocyte count, blood pressure and cardiovascular system have been noticed (Alexander, 1975; Goldcare et al., 1979; Petite et al, 1982, a b) which is in accordance with present findings in vasectomized monkeys. No alterations in haematocrit values were observed as in men by Pettit et al, (1982). Altered haemoglobin, RBC and haematocrit have been associated with androgen levels in males (Gardner et al., 1968) No alteration was observed in present study suggests no possibility of endocrine disorder as also observed by Goldcare et al., (1979) in men.

In vasectomized langur monkeys no change in standard haematological indices was observed. Haematological indices are good indicators of borderline of early phases of anemia (Lynch et al., 1969). Unaltered CI, MCV, MCH, MCHC and MCAT in present study suggest no possibility of anemia. Pettit et al (1982a) in men also reported the same observation. No change in total WBC count was observed which is in agreement with the findings of Alexander et al., (1974) Petitti et al., (1982) indicating no inflammatory reaction in the subject.

No alteration in any of the haematological measures in the present study suggests that vasectomy is not related to any disease state, as haematological tests form the very front line investigations on which diagnosis of many diseases is based (Goldcare et al., 1978; Walker et al., 1981 a b; WHO, 1982)

No alteration was observed in blood urea of vasectomized monkeys though it has been suggested that the vasectomy could result in higher uric acid levels due to increased resorption of nucleic acids (Johnson, 1972). Ansbacher (1973) and Petitti et al., (1982a) reported that in 6 years to more than 10 years vasectomized men no alteration in blood urea was observed and thus suggested that vasectomy is not associated with nucleic acid metabolism as in langur monkeys. No alteration in Alkaline phosphatase was observed in the present findings in langur monkeys as in 13 years vasectomized rhesus monkeys (Alexander and Tung (1979) and men Petitte et al (1982) Lactic dehydrogenase, transaminases (SGOT and SGPT) the indicators of hepatic and cardiac disorders remained unaltered in the present findings as observed by Alexander and Tung (1979) and Petitte et al (1982) in ten or more years vasectomized men.

In the present study no alteration was observed in SGOT and SGPT levels (Alexander et al., 1979) indicator of cardiac and hepatic disorders suggesting that vasectomy is not associated with heart and liver disorders in subjects (Goldcare et al., 1979, Walker et al., 1981)

Enhanced plasma cholesterol has been reported in the atherogenic diet induced and normal vasectomized cynomologus monkeys (WHO, 1982) and rabbits (Quigley et al., 1982) suggesting an increased possibility of atherosclerosis in vasectomized subjects. In the present study no significant alteration in total cholesterol, total lipid and phospholipids constituents at any of the intervals examined following vasectomy was observed. Vasectomy does not cause any

change in lipid metabolism, resulting in any possibility of atherosclerosis in langur monkeys. No change in sodium and potassium levels in vasectomized langur was observed as in rhesus monkey by Alexander and Tung (1979). Nienhuis(1992) also reports no association of Vasectomy with an increased risk of testicular cancer or the other diseases studied. Manson et al., (1999) provide reassuring evidence that vasectomy does not materially increase the risk of subsequent cardiovascular disease, even 15 or more years following the procedure.

Determination of serum bilirubin is useful in diagnosis of Jaundice where the value may increase 10 to 20 fold. In the present investigation no alteration was observed in the levels of bilirubin.

No long-term elevation of risk following vasectomy of asthma, diabetes mellitus, ankylosing spondylitis, thyrotoxicosis, multiple sclerosis, myasthenia gravis, inflammatory bowel disease, rheumatoid arthritis or testicular atrophy was observed in 13 years or more vasectomized men (Goldacre et al., 2007)

### CONCLUSION:

Vasectomy is considered to be safe in long and short terms. An impressive record of epidemiologic research over 15 major studies involving thousands of men with vasectomies are not at greater risk of any chronic illness than men who have not undergone the procedure.

The results of the present study provide no evidence that vasectomy leads to any such complications related to general health in langur monkeys (Goldcare et al., 2007; 1978; Petite et al., 1982; Walker et al., 1981).

### REFERENCES:

1. Alexander, NJ and Anderson, DJ. Vasectomy: Consequences of autoimmunity to sperm antigens. *Fertil. Steril.* 32, 253-260 (3) (1979).
2. Alexander, NJ and Tung, KSK. Effects of vasectomy in rhesus monkeys. In *Vasectomy: Immunologic and pathophysiologic effects in animals and man*. Lepow IH and Crozier, R. (eds). Acad. New York Press (1979) pp. 423-458.
3. Alexander, NJ. Antisperm antibodies in the rhesus after vasectomy and reanastomosis. *Abst. from VII Ann. Mtg. Soc. for the study of reproduction* (1974) pp. 174-175.
4. Alexander, NJ. Immunologic and morphologic effects of vasectomy in the rhesus monkey. *Fed. proc.* 34 (8), 1692-1697 (1975).
5. Anasbacher, R. Vasectomy: Sperm antibodies. *Fertil. Steril.* 24, 788-792 (1973).
6. Archer, H.E. and Robb, G.D. The determination of urea using urease. In *Colorimetric chemical analysis. The tintometer Ltd. Salisbury, England.* (1967). pp. 369-364.
7. Cabaud, P.G. and Wroblewski, F. Colorimetric measurement of lactic dehydrogenase activity of body fluids. *Am. J. Clin. Pathol.* 30, 234-236 (1958).
8. Crosby, W.H., Munn, J.I. and Furth, F.W. Standardizing a method for clinical haemoglobinometry. *U.S. Armed Forces Med. J.* 5, 695-703 (1954).
9. Gardner, F.H., Nathan, D.G., Piomelli, S. and Cumins, J.F. The erythrocythaemic effects of androgen. *Brit. J. Haem.* 14, 611-615 (1968).
10. Goldacre MJ, Wotton CJ, Seaqroatt V, Yeates D. Immune-related disease before and after vasectomy: an epidemiological database study. *Hum Reprod*22(5): 1273-8 (2007),
11. Goldcare, M.J., Clarke, J.A., Haesman, M. and Vessey, M.P. Follow-up vasectomy using medial record linkage. *Am. J. Epidemiol.* 108, 176-180 (1978).
12. Goldcare, M.J., Vessey, M.P., Clarke, J.A. and Haesman, M. Record linkage study of morbidity following vasectomy. In *Vasectomy: Immunologic and pathophysiologic*

- effects in animals and man*. Lepow, I.H. and Crozier, R. (eds.) Academic Press, New York (1979) pp. 567-575.
13. John, A.D. *Flame Photometry*. McGraw Hill Book Co. Inc. 295 (1960).
  14. Johnson, D.S. Reversible male sterilization: Current status and future directions. *Contraception*. 5, 327-338 (1972).
  15. King, E.J. and Coxon, V.J. Determination of bilirubin with precipitation of the plasma proteins. *J. Clin. Pathol.* 3, 248-259 (1950).
  16. Lynch, M.J., Raphael, S.S., Mellor, L.D., Spare, P.D. and Inwood, M.J.H. (eds.) *Medical Laboratory technology and clinical pathology*. W.B. Saunders Co., Philadelphia, London (1969) pp. 619-812.
  17. Manson JE, Ridker PM, Spelsberg A, Ajani U, Lotofo PA Hennekens CH Vasectomy and subsequent cardiovascular disease in US physicians. *Contraception*. 59 (3): 181-6 (1999)
  18. Massey, F.J. Jr., Bernstein G.S. and O'Fallon, W.M. Vasectomy and health: Results from a large cohort study. *J. Am. Med. Assoc.* 252 (8), 1023-1029 (1984).
  19. Natelson, S. Routine use of ultramicro-methods in the clinical laboratory. *Am. J. Clin. Pathol.* 21, 1153-1172 (1951).
  20. Nihuis H, Goldacre M, Seaqroatt V, Gill L, Vessey M (1992) 21 : 304 (6829) : 743-6 BMJ E Pub.
  21. Petitti, D.B., Klien, R., Kipp, H., and Friedman, G.D. Vasectomy and the incidence of hospitalized illness. *Br. J. Urol.* 129(4), 760-762. (1983).
  22. Petitti, D.B., Klien, R., Kipp, H., Kahn, W., Siegelau, A.B. and Friedman, G.D. Physiologic measures in men with and without vasectomies. *Fertil. Steril.* 37 (3), 438-330 (1982).
  23. Quigley, H.J., Lacy, S. and Curtis, G. Atherosclerosis in vasectomized rabbits. *Fertil. Steril.* 37, 321 (Abst.) (1982).
  24. Reitman, S. and Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* 28, 56-63 (1957).
  25. Walker, A.M., Jick, H., Hunter, J.R., Danford, A. and Rothman, K.J. Hospitalization rates in vasectomized men. *JAMA* 245, 2315-2317 (1981).
  26. Walker, M.W., Jick, H., Hunter, J.R., Danford, A., Walkins, R.N. and Alhadeff, L. and Vasectomy and non-fatal myocardial infarction. *The Lancet* 3, 13-15 (1981).
  27. WHO, Special programme of research, development and research training in human reproduction report of meeting held 3-6 Aug. 1981 at Geneva. *Contraception* 25(2), 119-123 (1982).