

# ·Original Article·

# Safety evaluation of long-term vas occlusion with styrene maleic anhydride and its non-invasive reversal on accessory reproductive organs in langurs

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# Abstract

Aim: To evaluate the safety of the long term vas occlusion with styrene maleic anhydride (SMA) and its non-invasive reversal at the level of accessory reproductive glands (ARGs) in langurs. Methods: The morphology of seminal vesicle and ventral prostate was evaluated by light as well as transmission electron microscopy. Serum clinical chemistry and urine albumin were evaluated in an autoanalyzer using reagent kits. Fructose, acid phosphatase and zinc in the seminal plasma were evaluated spectrophotometrically according to the WHO manual. Serum testosterone, prostate specific antigen and sperm antibodies were evaluated by enzyme-linked immunosorbent assays (ELISA) using reagent kits and hematology was estimated according to standard procedures. Results: The morphological features and secretory activity of the seminal vesicle and prostate were normal as evidenced by the presence of welldeveloped mitochondria, rough endoplasmic reticulum, Golgi bodies, secretory granules and normal nuclear characteristics throughout the course of investigation. Serum testosterone and prostate specific antigen remained unaltered and serum antisperm antibodies level presented negative titres. Urine albumin was nil. Total red blood corpuscles (RBC), white blood corpuscles (WBC), hemoglobin (Hb) and red cell indices, serum protein, glucose, cholesterol, creatinine, creatine kinase (CK), serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), lactate dehydrogenase (LDH), bilirubin, urea, triglycerides and high-density lipoprotein (HDL) did not show appreciable changes following vas occlusion and after its non-invasive reversal. Although fructose, acid phosphatase (ACP) and zinc in the seminal plasma showed a significant reduction following vas occlusion, it could not be related to the morphology of seminal vesicle and prostate. Conclusion: SMA vas occlusion and its non-invasive reversal do not damage the accessory reproductive organs. (Asian J Androl 2005 Jun; 7: 195-204)

Keywords: langurs; male contraception; poly styrene maleic anhydride

# 1 Introduction

In vas-based contraceptive methods, the status of

Correspondence to: Prof. N. K. Lohiya, Ph.D., Reproductive Physiology Section, Department of Zoology, University of Rajasthan, Jaipur – 302 004, India. Tel/Fax: +91-141-270-1809 E-mail: lohiyank@hotmail.com Received 2004-06-08 Accepted 2004-11-15 accessory reproductive glands, especially the seminal vesicle and the prostate, are often being neglected [1]. Because almost all the vas-based contraceptives currently on trial are keeping reversal in mind, greater attention needs to be focused on the accessory organs distal to the site of vas manipulation. Intravasal injectable contraceptive using styrene maleic anhydride (SMA) is a recent development among vas-based contraceptives, has completed Phase I and Phase II Clinical Trials [2, 3] and it is currently under Phase III Clinical Trial [4]. SMA is a combination of two monomers, the styrene and maleic anhydride in 1:2 ratio, has a well-established pH-lowering effect in the internal milieu of the vas, and has the positive charge which disturbs the negative charge of the plasma membrane of the spermatozoa that pass through the vas lumen [5]. The procedure has several advantages than any other vas-based contraceptives in that it is non-sclerotic [5], offers instant sterility [6] and could be administered by non-invasive no-scalpel injection [3]. In langurs, reversal of short term vas occlusion with SMA through a non-invasive procedure, involving palpation, percutaneous electrical stimulation, forced vibratory movements, suprapubic percussion in the vas segment and per rectal digital massage in the ampulla of the vas [7], and the feasibility of repeated vas occlusion and non-invasive reversal, have been successfully demonstrated [8].

Studies on the long-term sequel of vas occlusion with SMA and its reversal at the level of spermatogenesis, sperm maturation, sperm antibodies, status of accessory reproductive glands (ARGs) and serum hormones, are currently in progress in langurs (Presbytis entellus entellus Dufresne), as several of these issues are more difficult to study in humans because of ethical reasons. The longterm sequel of SMA vas occlusion for 540 days at the level of spermatogenesis has already been reported [9]. In the present investigation, the long-term safety of the SMA vas occlusion at the level of morphological status of accessory reproductive glands, seminal plasma biochemistry, serum hormones and sperm antibodies, urine albumin, routine hematology and serum clinical biochemistry up to 540 days of vas occlusion, and 150 days of non-invasive reversal, has been reported.

# 2 Materials and methods

#### 2.1 Animals

Adult male langurs, 6–7-year-old, as identified by musculature, sexual skin in the rump and dentition [10] and weighing 12–15 kg were used in the present investigation. They are non-seasonal breeders and show closer association to humans in reproductive exocrine and endocrine profiles [11]. The animals were trapped in and around Jaipur and kept in quarantine for a period of 3 months in individual metallic cages and fed with seasonal vegetables, fruits and wheat cakes; water was provided *ad libitum*. The health and reproductive status

of the animals was assessed through routine semen analysis, hematology and clinical chemistry and only healthy animals were used in the present investigations. The experiments were conducted in accordance with the 'Guidelines for Care and Use of Animals in Scientific Research, Indian National Science Academy, New Delhi, 2000' and the entire protocol has the approval of Institutional Ethical Committee, University of Rajasthan, Jaipur, India.

#### 2.2 Pretreatment sampling

Prior to vas occlusion, at least three pretreatment semen samples were collected by penile electro-stimulation for semen biochemistry, and blood samples were collected through saphenous veins for hematology, serum clinical biochemistry, serum testosterone, sperm antibodies and prostate specific antigen.

#### 2.3 Surgical procedures

#### 2.3.1 Vas occlusion

Ten animals were injected with 60 mg of SMA dissolved in 120  $\mu$ L dimethylsulfoxide (DMSO) into the lumen of each vas deferens, exposed closer to the external inguinal segment, under sodium thiopentone anesthesia (20 mg/kg, i.v.) with the flow directed towards the ampulla avoiding retrograde flow. Three animals served as the sham operated vehicle treated (120  $\mu$ L DMSO) control. Postoperative care was provided with antibiotic and anti-inflammatory drugs and all the animals had uneventful postoperative recovery [7].

#### 2.3.2 Non-invasive reversal

Non-invasive reversal procedure was performed in seven of the ten vas occluded animals under anesthesia after 540 days of vas occlusion. The technique, carried out in progressive course from the scrotal to inguinal canal, aimed to propel the SMA from the vas deferens to the ejaculatory duct. It involved palpation, percutaneous squeezing of the vas deferens at the scrotal and inguinal segments, electrical stimulation (40 V) and vibration through a specially designed vibrator at the external inguinal segment, supra-pubic percussion with rubber hammer and per-rectal digital massage to the ampullary segment of the vas deferens in successive steps. The procedures were repeated three to four times and the entire operation took 15–20 minutes [7]. The animals did not show any sign of discomfort after the reversal procedure.

#### 2.4 Seminal plasma biochemistry

Seminal plasma, free of spermatozoa and obtained by centrifugation, was used for the monthly estimation of fructose, acid phosphatase (ACP) and zinc up to 540 days of vas occlusion and 150 days of reversal. Fructose and ACP were estimated according to the WHO manual [12]. Zinc was estimated calorimetrically using a reagent kit (Wako Pure Chemical Industries Ltd., Japan).

## 2.5 Hematology

Total red blood corpuscles (RBC), white blood corpuscles (WBC), hemoglobin (Hb), red cell indices viz., packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated monthly following vas occlusion for 540 days and its non-invasive reversal for 150 days [13–15].

#### 2.6 Serum clinical biochemistry

Serum protein, glucose, cholesterol, creatinine, creatine kinase (CK), serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), lactate dehydrogenase (LDH), bilirubin, urea, triglycerides and high-density lipoprotein (HDL) were estimated colorimetrically monthly up to 540 days of vas occlusion and 150 days of reversal using reagent kits (Transasia Biomedicals Ltd., Mumbai, India).

#### 2.7 Testosterone, ASA and PSA levels

Serum testosterone (EIA Kit: Biochem Immuno Systems, Italy), prostate specific antigen (EIA Kit: Quadratech Diagnostics Ltd., UK) and sperm antibodies (ELISA Kit: Bioserv AG, Germany) were assayed periodically up to 540 days of vas occlusion and after 150 days of reversal.

#### 2.8 Urine analysis

Urine samples were collected quarterly in sterile vials for estimation of albumin to identify if vas occlusion with SMA had any effect on albuminuria (Prof. S. K. Guha, personal communication).

## 2.9 Morphology of accessory reproductive glands (ARGs)

The seminal vesicle and prostate samples were obtained under sodium thiopentone anesthesia after 540 days of vas occlusion and 150 days of reversal.

# 3 Results

3.1 Morphology of ARGs

#### 3.1.1 Seminal vesicle

#### 3.1.1.1 Histology

The seminal vesicle of the control animals consisted of cuboidal epithelium thrown into crypts. The epithelial mucosa was surrounded by longitudinal muscle layers. Adjacent to the muscular layer, the epithelial crypts became deeper and formed smaller follicles. The crypts were elongated and showed deep indentations. The lumen contained eosinated secretory material. The peripheral region was made up of smaller compartments and looked like a tubular lumen surrounded by cryptic epithelium. The epithelial cells contained centrally placed nucleus and granular cytoplasm (Figure 1). Vas occlusion with SMA for 540 days and its non-invasive reversal for 150 days did not show appreciable changes in the histology of seminal vesicle (Figure 2).

#### 3.1.1.2 Ultrastructure

Vas occlusion for 540 days or following 150 days of its reversal did not show appreciable changes in the ultrastructure of the seminal vesicle compared to that of the control animals. The nuclei were round or oval with a double membranous structure with patchy chromatin material and occasional nucleolus. The cytoplasm was occupied with cellular organelles characteristic of secretory functions including well-defined mitochondria, Golgi bodies, rough endoplasmic reticulum and secretory granules (Figures 3 and 4).

# 3.1.2 Prostate

#### 3.1.2.1 Histology

The prostate of the controls was made up of several lobules, each consisting of the typical prostatic follicle with a definite lumen. Each lobule was occupied by a single large follicle or three or more smaller follicles. Interlobular region was occupied with connective tissue. The epithelial cells were tall columnar containing the nucleus placed at the base or centre of the cells. The cytoplasm appeared granular and the lumen was filled with secretory material (Figure 5).

There were no appreciable changes observed in the histology of prostate following 540 days of vas occlusion or after 150 days of non-invasive reversal (Figure 6).



Figure 1. Histology of seminal vesicle of control animals showing the cuboidal epithelium arranged in crypts. Additionally, few follicles (F) are evident in the basal region. The epithelium is surrounded by a longitudinal muscle layer ( $\times$ 100). MU, muscle layer.



Figure 2. Histology of seminal vesicle following 540 days of vas occlusion with styrene maleic anhydride (SMA). Note that the epithelial features are similar to that of controls (×100). LU, lumen.



Figure 3. Ultrastructure of seminal vesicle following 540 days of vas occlusion with SMA. The nucleus (N) shows a distinct nuclear membrane and patchy chromatin material. A single nucleolus (NU) is also present in the nucleoplasm. The cytoplasm is rich with secretory granules (SG) and rough endoplasmic reticulum (RER) (×5600).

# 3.1.2.2 Ultrastructure

Following 540 days of vas occlusion, there were no appreciable changes observed in the secretory activity of the prostatic cells compared to that of control animals. Few of the cells depicted vacuolized mitochondria and electron transparent vesicles; Golgi bodies and stacks of endoplasmic reticulum were prominent. Free ribosomes and secretory granules were scattered throughout the



Figure 4. Ultrastructure of seminal vesicle after 150 days of noninvasive reversal showing the supra nuclear region. The cytoplasm contains secretory granules (SG), lysosomes (LY) and multi-vesicular bodies (MVB) (×5600).

cytoplasm. The nuclei were generally oval, however, and in a few of the cells the nuclei showed an irregular pattern (Figure 7).

After 150 days of reversal, the ultrastructural features represented the typical cells with an oval nucleus and well-developed cytoplasmic organelles (Figure 8).

## 3.2 Seminal plasma biochemistry



Figure 5. Histology of prostate of control animals. Prostate is made up of several lobules containing typical prostatic follicles (F) with definite lumen. Connective tissues (C) occupied the entire lobular region. Epithelium is tall columnar and cryptic (×100).



Figure 6. Histology of prostate following 540 days of vas occlusion with SMA. Note that histological features are similar to that of controls ( $\times 100$ ).



Figure 7. Ultrastructure of prostate following 540 days of vas occlusion with SMA showing vesicular cytoplasm. Secretory granules (SG) however appear prominent. Golgi bodies and rough endoplasmic reticulum are well-defined (×5600).

The fructose, ACP and zinc in the seminal plasma showed a gradual significant reduction (P < 0.05 to P < 0.001) beginning 30–90 days of vas occlusion that continued until after 540 days of the vas occlusion period. Non-invasive reversal resulted in the recovery to pretreatment values after 150 days (Figure 9).

# 3.3 Hematology



Figure 8. Ultrastructure of the prostate after 150 days of noninvasive reversal showing well-developed nucleus (N) with distinct nuclear membrane (NM) and patchy chromatin material. Cytoplasm adjacent to nucleus is characterized by secretory granules (SG) and coated vesicles (CV). Plasma membrane (P) shows irregular patterning (×7200).

Total RBC, WBC, Hb, PCV, MCV, MCH and MCHC did not show appreciable changes following vas occlusion up to 540 days and 150 days of non-invasive reversal (Table 1).

#### 3.4 Clinical biochemistry

The serum levels of total protein, glucose, cholesterol, creatinine, SGPT, SGOT, LDH, CK, bilirubin, urea, TGL,

HDL and LDH following vas occlusion up to 540 days, and after its non-invasive reversal up to 150 days, did not show appreciable changes, although showed minor fluctuations (Table 2).

# 3.5 Testosterone, ASA and PSA levels

The serum testosterone, ASA and PSA levels following 150, 300, 450 and 540 days of vas occlusion and after its non-invasive reversal, did not show appreciable



Figure 9. Semen biochemistry of langur monkeys prior to and following vas occlusion with SMA (n=10) and after its non-invasive reversal (n=10). PT, pretreatment SG.  ${}^{b}P < 0.05$ ,  ${}^{c}P < 0.01$ , compared with PT.

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Treatment				Parameters			
Schedule	RBC	WBC	Hemoglobin	PCV	MCV	MCH	MCHC
	$(10^{6}/\text{mm}^{3})$	(10 <sup>3</sup> /mm <sup>3</sup> )	(g/dL)	(%)	(µm <sup>3</sup> )	(μ/μg)	(%)
Pretreatment*	$5.2 \pm 1.2$	$12.3\pm0.9$	$12.2\pm0.1$	$40.9\pm1.6$	$72.0\pm2.6$	$22.1\pm0.6$	$33.8\pm0.3$
Treatment*							
30 days	$5.4 \pm 0.2$	$11.5\pm0.4$	$12.1\pm0.2$	$41.0\pm1.0$	$71.8\pm2.8$	$23.0\pm0.6$	$32.2\pm0.9$
60 days	$5.5\pm0.1$	$11.8\pm0.4$	$12.4\pm0.4$	$40.1\pm1.3$	$73.3\pm3.4$	$22.5\pm0.8$	$31.3\pm1.6$
90 days	$5.2\pm0.2$	$12.6\pm0.3$	$11.9\pm0.3$	$38.7 \pm 1.1$	$74.1\pm3.0$	$22.7\pm0.6$	$31.3 \pm 1.3$
120 days	$5.2\pm0.1$	$12.4\pm0.3$	$12.0\pm0.3$	$39.1 \pm 1.0$	$75.1 \pm 2.2$	$23.0\pm0.4$	$30.6\pm2.1$
150 days	$5.1 \pm 0.2$	$11.9\pm0.0$	$12.3\pm0.4$	$40.3\pm1.6$	$79.0\pm3.2$	$24.1 \pm 1.3$	$30.5\pm2.3$
180 days	$5.0\pm0.2$	$12.3\pm0.1$	$11.8\pm0.2$	$40.2\pm1.4$	$80.4\pm4.4$	$23.6\pm0.9$	$29.3\pm2.8$
210 days	$5.2\pm0.2$	$11.5\pm0.1$	$11.9\pm0.2$	$39.4 \pm 1.7$	$75.7\pm3.7$	$22.8\pm0.9$	$30.2\pm2.9$
240 days	$5.1\pm0.2$	$11.7\pm0.0$	$12.1\pm0.4$	$38.1\pm0.7$	$74.7\pm4.2$	$23.7\pm2.0$	$31.7 \pm 1.2$
270 days	$5.3 \pm 0.3$	$11.8\pm0.2$	$12.3\pm0.4$	$40.8\pm0.9$	$76.9\pm3.8$	$23.2\pm2.3$	$30.1\pm2.0$
300 days	$5.1 \pm 0.2$	$12.4\pm0.2$	$12.0\pm0.4$	$39.7\pm0.1$	$77.8\pm3.0$	$23.5\pm1.8$	$30.2\pm2.6$
330 days	$4.9\pm0.1$	$12.1\pm0.2$	$11.7\pm0.2$	$40.5\pm0.6$	$82.6\pm6.0$	$23.8 \pm 1.9$	$28.8 \pm 4.0$
360 days	$5.1 \pm 0.1$	$12.2\pm0.3$	$11.5\pm0.2$	$41.3\pm1.6$	$80.9\pm4.8$	$22.5\pm0.8$	$27.8\pm4.1$
390 days	$5.2\pm0.3$	$11.9\pm0.5$	$11.8\pm0.3$	$40.1\pm2.5$	$77.1 \pm 4.5$	$22.6 \pm 1.9$	$29.4\pm2.7$
420 days	$5.3\pm0.3$	$11.5\pm0.5$	$12.0\pm0.4$	$39.6\pm2.2$	$74.7\pm4.6$	$22.6 \pm 1.0$	$30.3\pm2.7$
450 days	$5.4 \pm 0.4$	$11.2\pm0.5$	$12.2\pm0.2$	$38.9\pm2.5$	$72.0\pm5.0$	$22.5\pm0.6$	$31.3\pm2.1$
480 days	$5.0\pm0.3$	$11.6\pm0.3$	$12.1\pm0.2$	$38.1 \pm 1.1$	$76.2\pm2.3$	$24.2\pm1.8$	$31.7 \pm 1.7$
510 days	$4.9\pm0.2$	$11.8\pm0.4$	$11.9\pm0.2$	$39.1 \pm 1.4$	$79.7\pm3.8$	$24.2\pm2.0$	$30.4\pm2.4$
540 days	$5.1 \pm 0.1$	$11.4\pm0.3$	$12.3\pm0.1$	$38.7 \pm 1.6$	$75.8 \pm 1.5$	$24.1 \pm 1.6$	$31.7\pm1.3$
Reversal <sup>#</sup>							
0 days	$4.9\pm0.2$	$11.9\pm0.2$	$11.8\pm0.4$	$39.2\pm1.5$	$80.0\pm4.4$	$24.0\pm1.5$	$30.1\pm2.6$
10 days	$5.2 \pm 0.4$	$12.3\pm0.1$	$12.0\pm0.4$	$40.6\pm1.3$	$78.0\pm2.6$	$23.0\pm0.8$	$29.5\pm2.6$
20 days	$5.3\pm0.2$	$12.0\pm0.2$	$11.6\pm0.3$	$39.3 \pm 1.4$	$68.9\pm3.3$	$21.8\pm0.5$	$29.5\pm3.0$
30 days	$5.1\pm0.3$	$12.4\pm0.6$	$11.7\pm0.3$	$39.5\pm2.1$	$77.4\pm3.5$	$22.9\pm0.8$	$29.8\pm2.6$
60 days	$5.0\pm0.2$	$11.7\pm0.5$	$12.0\pm0.4$	$38.2\pm2.2$	$76.4\pm3.9$	$24.0 \pm 1.4$	$31.5\pm1.4$
90 days	$5.4\pm0.1$	$11.8\pm0.4$	$12.2\pm0.2$	$41.6\pm0.9$	$77.1\pm2.4$	$22.6\pm0.4$	$30.0\pm0.6$
120 days	$5.2\pm0.2$	$11.9\pm0.4$	$11.8\pm0.2$	$39.7 \pm 1.6$	$76.1\pm3.3$	$22.7\pm0.5$	$29.8 \pm 1.4$
150 days	$5.0 \pm 0.2$	$12.2\pm0.2$	$12.0\pm0.2$	$38.9 \pm 1.5$	$76.7\pm3.1$	$23.4\pm1.0$	$29.1 \pm 1.4$

Table 1. Haematological parameters of langur monkeys prior to and following vas occlusion with SMA and after its non-invasive reversal.

\* - Values are SEM of 10 animals; # - Values are SEM of 7 animals

changes. ASA levels remained in a negative range throughout the study period (normal values 0-60 U/mL) (Table 3).

# 3.6 Urine analysis

Urine albumen that was analyzed quarterly during pre-treatment, following vas occlusion up to 540 days of the study period and after its non-invasive reversal up to 150 days, remained as a trace or nil, and thus revealed negative albuminuria throughout the study period.

# 4 Discussion

In traditional vasectomy, the long term health consequences have been of great concern to the general public and to health workers. Currently, vas occlusion with SMA has been proven to be the one that meets all the essential criteria of a male contraceptive [1, 16]. Preliminary studies in langurs suggested instant oligoasthenoteratozoospermia and subsequent uniform azoospermia up to 540 days [6, 9]. Instant azoospermia reversal on the same day of reversal manipulation, and

#### Safety evaluation of styrene maleic anhydride

Treatment				Parameters		
Schedule	Protein	Glucose	Cholesterol	Creatinine	SGPT	SGOT
	(mg/dL)	(mg/dL)	(mg/dL)	(mg/dL)	(IU/L)	(IU/L)
Pretreatment*	$6.2\pm0.2$	$102.7\pm3.7$	$170.0\pm3.6$	$2.4\pm0.2$	$3.2\pm0.2$	$3.2 \pm 0.3$
Treatment (days)*						
30	$6.3 \pm 0.1$	$102.9 \pm 1.5$	$171.5\pm3.8$	$2.3\pm0.1$	$3.2 \pm 0.0$	$3.0\pm0.1$
60	$6.2\pm0.1$	$101.4 \pm 1.2$	$172.7\pm3.2$	$2.2\pm0.1$	$3.2\pm0.1$	$3.1\pm0.1$
90	$6.0 \pm 0.1$	$100.2\pm1.1$	$173.2\pm3.8$	$2.2\pm0.1$	$3.1 \pm 0.0$	$3.0\pm0.1$
120	$5.9\pm0.1$	$98.6 \pm 1.0$	$170.4\pm3.9$	$2.2\pm0.1$	$3.1 \pm 0.1$	$2.8\pm0.1$
150	$5.8 \pm 0.1$	$98.4\pm0.9$	$162.3\pm4.1$	$2.1\pm0.1$	$2.9\pm0.1$	$2.9\pm0.1$
180	$5.7\pm0.2$	$99.4 \pm 1.0$	$169.6\pm4.7$	$2.0\pm0.2$	$3.0\pm0.1$	$3.0\pm0.1$
210	$5.8\pm0.2$	$100.2\pm0.4$	$165.6\pm4.8$	$2.3\pm0.0$	$3.2\pm0.2$	$3.2\pm0.1$
240	$5.9 \pm 0.2$	$99.0\pm2.1$	$162.0\pm3.2$	$2.1 \pm 0.1$	$3.1 \pm 0.3$	$3.2\pm0.2$
270	$6.0 \pm 0.1$	$103.0\pm1.0$	$170.1\pm3.3$	$2.2\pm0.1$	$2.9\pm0.3$	$3.1 \pm 0.1$
300	$6.1 \pm 0.1$	$99.5\pm0.9$	$163.5\pm3.8$	$2.1 \pm 0.1$	$2.8\pm0.2$	$2.9\pm0.1$
330	$6.3 \pm 0.3$	$96.8 \pm 1.0$	$169.8\pm3.3$	$2.0 \pm 0.1$	$2.8\pm0.2$	$2.8\pm0.1$
360	$6.2 \pm 0.2$	$107.0\pm0.9$	$167.6\pm2.0$	$2.0\pm0.1$	$2.9\pm0.1$	$3.0 \pm 0.1$
390	$5.7 \pm 0.2$	$98.5\pm3.7$	$167.2\pm8.9$	$2.2 \pm 0.1$	$2.7 \pm 0.3$	$3.0 \pm 0.2$
420	$5.7 \pm 0.2$	$96.4\pm4.8$	$168.6\pm9.0$	$2.1 \pm 0.1$	$2.8 \pm 0.3$	$2.9\pm0.2$
450	$5.9\pm0.3$	$102.7\pm4.6$	$173.2\pm7.8$	$2.3\pm0.0$	$3.0 \pm 0.2$	$2.7\pm0.2$
480	$5.8 \pm 0.2$	$97.3 \pm 4.2$	$171.6\pm6.4$	$2.4 \pm 0.1$	$3.1 \pm 0.2$	$3.1 \pm 0.1$
510	$6.1\pm0.2$	$96.7\pm2.2$	$174.8 \pm 4.2$	$2.2\pm0.0$	$2.9 \pm 0.4$	$2.7\pm0.4$
540	$5.8\pm0.2$	$95.3\pm3.2$	$170.5\pm3.2$	$2.2\pm0.1$	$2.8\pm0.2$	$2.9\pm0.1$
Reversal (days)#						
0	$6.2 \pm 0.2$	$98.7 \pm 1.1$	$171.4\pm0.1$	$2.4 \pm 0.1$	$2.7\pm0.3$	$2.7\pm0.4$
10	$5.9\pm0.2$	$100.3\pm2.2$	$174.2\pm3.0$	$2.1 \pm 0.1$	$2.9\pm0.3$	$2.8\pm0.4$
20	$5.8\pm0.1$	$98.8 \pm 1.3$	$176.8\pm2.8$	$2.3\pm0.0$	$3.0\pm0.1$	$2.9\pm0.2$
30	$6.2\pm0.2$	$99.8\pm4.8$	$177.1\pm7.9$	$2.2\pm0.1$	$3.2\pm0.1$	$3.0 \pm 0.2$
60	$6.3\pm0.2$	$102.0\pm4.8$	$179.2\pm7.7$	$2.3\pm0.1$	$3.0\pm0.2$	$3.1\pm0.2$
90	$6.5\pm0.1$	$103.9\pm2.6$	$173.9 \pm 1.0$	$2.4\pm0.2$	$3.2 \pm 0.1$	$3.2 \pm 0.1$
120	$6.3\pm0.1$	$100.1\pm1.6$	$155.5\pm8.4$	$2.5\pm0.2$	$3.1\pm0.1$	$3.1 \pm 0.1$
150	$6.3\pm0.2$	$99.1 \pm 1.7$	$165.4\pm4.5$	$2.4\pm0.1$	$2.9\pm0.2$	$3.2\pm0.2$

Table 2. Serum clinical biochemistry of langur monkeys prior to and following vas occlusion with SMA and after its non-invasive reversal.

\* - Values are SEM of 10 animals; # - Values are SEM of 7 animals

normozoospermia within 3–5 ejaculations, were also well established [7, 8]. In the present investigation, we have attempted to elucidate the structural details of the seminal vesicle and prostate in langurs following 540 days of vas occlusion with SMA and 150 days of non-invasive reversal at the level of histology and ultrastructure along with the seminal plasma markers of these organs, so as to establish the safety of the procedure at the level of ARGs and also to eliminate the associated risks that have arisen in vasectomy, amidst controversies [17] that the procedure could lead to prostatic carcinoma or other disorders. (To be continued)

The structural details as obtained in the present investigation indicated that there is no associated risk of the procedure with that of the malfunction of the ARGs, particularly in relation to prostate. The morphological features of seminal vesicle and prostate presented normal secretory activity even after 540 days of vas occlusion [18]. The unaltered levels of serum testosterone and PSA in the present investigation further confirm this theory. However, the reasons for the decline in fructose, ACP and zinc in the seminal plasma remain unknown, but they could not be related to the functional status of ARGs as the serum testosterone, PSA and ultrastruc-

Treatment				Parameters			
Schedule	LDH	СК	Bilirubin	Urea	TGL	HDL	LDL
	(I/L)	(IU/L)	(IU/L)	(mg/dL)	(mg/dL)	(mg/dL)	(mg/dL)
Pretreatment*	$31.8\pm2.4$	$53.2\pm3.9$	$25.2\pm1.9$	$43.1\pm1.5$	$151.4\pm5.5$	$102.0\pm2.7$	$50.1 \pm 4.7$
Treatment (days)*							
30	$29.8\pm0.5$	$53.1\pm1.0$	$23.7\pm0.8$	$40.8\pm1.1$	$160.1 \pm 1.7$	$99.5 \pm 1.0$	$50.0 \pm 4.7$
60	$29.8\pm0.5$	$51.7\pm1.0$	$24.0\pm1.1$	$42.0\pm0.9$	$154.9\pm5.0$	$98.1\pm0.4$	$49.7 \pm 3.2$
90	$30.2\pm0.7$	$49.8 \pm 1.2$	$22.6 \pm 1.1$	$41.3\pm1.5$	$161.6\pm4.5$	$97.6\pm0.8$	$51.2 \pm 3.4$
120	$30.6\pm0.5$	$49.0\pm1.6$	$21.8\pm0.9$	$40.3\pm1.5$	$162.4\pm4.8$	$97.1 \pm 1.0$	$48.9\pm4.2$
150	$29.3\pm0.7$	$49.0\pm1.5$	$23.3 \pm 1.7$	$42.4\pm2.3$	$153.2\pm9.2$	$97.8 \pm 1.2$	$46.3 \pm 5.0$
180	$29.4\pm0.4$	$48.5\pm1.9$	$21.0\pm1.6$	$39.3\pm2.3$	$168.6 \pm 10.7$	$96.9 \pm 1.5$	$41.2 \pm 6.1$
210	$29.2\pm0.6$	$49.7\pm2.2$	$25.0\pm0.8$	$41.8 \pm 1.9$	$141.9\pm8.6$	$97.4\pm2.8$	$37.5 \pm 6.4$
240	$28.6 \pm 1.7$	$49.0\pm2.9$	$24.1\pm3.2$	$39.6 \pm 1.2$	$150.1\pm8.6$	$96.5\pm2.6$	$41.1 \pm 5.0$
270	$28.3 \pm 1.8$	$51.2 \pm 3.4$	$23.6 \pm 1.5$	$38.5\pm2.5$	$156.6 \pm 10.5$	$96.0\pm2.8$	$47.7 \pm 5.8$
300	$29.0 \pm 1.2$	$49.1\pm3.0$	$21.5\pm1.8$	$39.1 \pm 1.9$	$164.9\pm8.9$	$98.4\pm3.8$	$44.7 \pm 7.7$
330	$29.6 \pm 1.0$	$49.5\pm1.6$	$24.0\pm0.3$	$40.6\pm1.7$	$168.2\pm9.5$	$95.4\pm5.6$	$49.6 \pm 8.5$
360	$30.0\pm1.0$	$50.8\pm2.1$	$25.3\pm0.9$	$42.9 \pm 1.7$	$165.0\pm8.2$	$96.3\pm5.5$	$52.7 \pm 4.8$
390	$31.2\pm1.0$	$49.2\pm3.3$	$23.7\pm0.6$	$42.1\pm1.0$	$160.6\pm3.2$	$98.3\pm2.6$	$50.9 \pm 0.5$
420	$29.2 \pm 1.0$	$48.7\pm3.1$	$22.8\pm0.8$	$39.4 \pm 1.9$	$163.0\pm4.3$	$99.9 \pm 1.8$	$48.8 \pm 0.6$
450	$30.8\pm0.9$	$50.6\pm3.5$	$22.3\pm0.5$	$38.7 \pm 1.8$	$161.6\pm4.7$	$98.1 \pm 1.9$	$44.9 \pm 0.5$
480	$29.1 \pm 1.2$	$49.1 \pm 1.8$	$23.1\pm0.8$	$39.1\pm2.0$	$160.1\pm5.7$	$97.9\pm2.5$	$46.7 \pm 1.2$
510	$28.5\pm1.0$	$47.3 \pm 1.5$	$24.6 \pm 1.3$	$40.3\pm1.3$	$164.6\pm7.2$	$96.1\pm4.6$	$46.9 \pm 1.1$
540	$30.1 \pm 1.6$	$46.9 \pm 1.6$	$25.7 \pm 1.2$	$41.9 \pm 1.2$	$169.9 \pm 10.0$	$95.3\pm6.0$	$47.1 \pm 1.2$
Reversal (days)#							
0	$27.3 \pm 1.5$	$49.7 \pm 1.0$	$24.5\pm2.2$	$40.1\pm1.7$	$161.7\pm3.2$	$97.5\pm2.8$	$47.3 \pm 1.6$
10	$27.7 \pm 1.7$	$47.5\pm1.7$	$23.1\pm1.6$	$39.7\pm2.2$	$164.5\pm7.1$	$99.7\pm2.1$	$51.5 \pm 2.4$
20	$28.0 \pm 1.1$	$48.6\pm2.4$	$22.9 \pm 1.2$	$39.8\pm2.5$	$159.3 \pm 1.4$	$97.3 \pm 1.3$	45.3 ± 2.7
30	$28.0\pm1.3$	$50.6 \pm 2.2$	$21.6\pm1.1$	$38.5 \pm 2.8$	$154.4\pm4.0$	$95.9\pm2.8$	$46.2 \pm 1.0$
60	$29.6\pm0.7$	$49.3 \pm 1.9$	$22.0\pm1.0$	$40.6\pm2.0$	$151.1\pm2.8$	$96.5\pm3.3$	$49.0 \pm 1.2$
90	$30.4\pm0.4$	$51.5\pm0.9$	$23.2\pm0.6$	$41.0\pm1.1$	$150.9 \pm 1.0$	$98.8 \pm 1.2$	$48.5 \pm 2.5$
120	$27.0\pm2.0$	$50.9 \pm 1.6$	$24.8\pm0.9$	$39.5 \pm 1.8$	$148.8 \pm 1.8$	$100.4 \pm 1.3$	$48.5 \pm 3.2$
150	$28.0 \pm 2.2$	$49.6 \pm 2.6$	$23.6 \pm 1.2$	$39.7 \pm 2.3$	$146.9 \pm 1.6$	$99.8 \pm 1.7$	$49.8 \pm 1.1$

\* - Values are SEM of 10 animals; # - Values are SEM of 7 animals

tural study indicated no drastic changes in the secretory activity of the seminal vesicle and prostate. Although it has been well-established that the langurs lack seasonality in their reproductive and breeding pattern, variation in semen volume during different seasons, has also been reported [11]. Thus, the role of variable climatic conditions in bringing out fluctuations in the biochemical composition of seminal plasma cannot be ruled out. Clinical safety evaluation of the RBC, WBC, Hb and red cell indices and serum protein, glucose, cholesterol, creatinine, CK, SGOT, SGPT, LDH, bilirubin, urea, triglycerides and HDL suggested unaltered physiological/metabolic activity. The presence of albuminuria, as questioned in the Phase III Clinical Trial (Prof. S. K. Guha, Personal Communication) was not observed in the present investigation. The negative titres of sperm antibodies is more favorable for the functional success of the reversal of the procedure. It is concluded that SMA vas occlusion and its non-invasive reversal do not damage the accessory reproductive organs.

Table 3. Serum Testosterone, ASA and PSA levels in langur monkeys prior to and following vas occlusion with SMA and after its non-invasive reversal.

Treatment	Paramaters						
Schedule	Testosterone	ASA	PSA				
	(ng/mL)	(U/mL)	(U/mL)				
Pretreatment*	$5.8 \pm 3.9$	$4.75\pm0.27$	$0.98\pm0.08$				
Treatment (days)*							
150	$6.1 \pm 2.3$	$4.83\pm0.23$	$0.94\pm0.07$				
300	$5.4 \pm 4.4$	$5.12\pm0.89$	$1.02\pm0.09$				
450	$6.2 \pm 3.3$	$4.92\pm0.37$	$0.99\pm0.08$				
540	$5.9 \pm 4.5$	$5.63 \pm 0.31$	$0.96\pm0.09$				
Reversal (days) #							
150	$6.3 \pm 3.2$	$4.87 \pm 0.21$	$1.01 \pm 0.05$				

\* - values are SEM of 10 animals; # - values are SEM of 7 animals.

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## References

- Lohiya NK, Manivannan B, Mishra PK, Pathak N. Vas deferens, a site of male contraception: an overview. Asian J Androl 2001; 3: 87–95.
- 2 Guha SK, Singh G, Anand S, Ansari S, Kumar S, Koul V. Phase I clinical trial of an injectable contraceptive for the male. Contraception 1993; 48: 367–75.
- 3 Guha SK, Singh G, Ansari S, Kumar S, Srivastava A, Koul V, *et al.* Phase II clinical trial of a vas deferens injectable contraceptive for the male. Contraception 1997; 56: 245–50.
- 4 Chaki SP, Das HC, Misro MM. A short-term evaluation of semen and accessory sex gland function in phase III trial subjects receiving intravasal contraceptive RISUG. Contracep-

tion 2003; 67: 73-8.

- 5 Guha SK. Contraceptive for use by a male. US Patent 54880705: 1996.
- 6 Lohiya NK, Manivannan B, Mishra PK. Ultrastructural changes in the spermatozoa of langur monkeys, *Presbytis entellus entellus* after vas occlusion with styrene maleic anhydride. Contraception 1998; 57: 125–32.
- 7 Lohiya NK, Manivannan B, Mishra PK, Pathak N, Balasubramanian SP. Intravasal contraception with styrene maleic anhydride and its noninvasive reversal in langur monkeys (*Presbytis entellus entellus*). Contraception 1998; 58: 119–28.
- 8 Lohiya NK, Manivannan B, Mishra PK. Repeated vas occlusion and non-invasive reversal with styrene maleic anhydride for male contraception in langur monkeys. Int J Androl 2000; 23: 36–42.
- 9 Mishra PK, Manivannan B, Pathak N, Sriram S, Bhande SS, Panneerdoss S, *et al.* Status of spermatogenesis and of sperm parameters in langur monkeys following long term vas occlusion with styrene maleic anhydride (SMA). J Androl 2003; 24: 501–9.
- 10 David GF, Ramaswami LS. Reproductive systems of the N orth Indian langur (*Presbytis entellus entellus* Dufresne). J Morphol 1971; 135: 99–129.
- 11 Lohiya NK, Sharma RS, Manivannan B, Anand Kumar TC. Reproductive exocrine and endocrine profiles and their seasonality in male langur monkeys (*Presbytis entellus entellus*). J Med Primatol 1998; 27: 15–20.
- 12 WHO. Laboratory Manual for Examination of Human Semen and Sperm cervical Mucus Interaction. New York: Cambridge University Press; 1992.
- 13 Crosby WH, Munn JI, Furth FW. Standardizing a method for clinical haemoglobinometry. US Armed Forces Med J 1954; 5: 693–703.
- 14 Lynch MJ, Raphael SS, Mellor LD, Spare PD, Inwood MJH. Medical Laboratory Technology and Clinical Pathology. London: WB Saunders Company; 1969.
- 15 Natelson S. Routine use of ultramicro methods in the clinical laboratory; estimation of sodium, potassium, chloride, protein, hematocrit value, sugar, urea and nonprotein nitrogen in fingertip blood; construction of ultramicro pipets; a practical microgasometer for estimation of carbon dioxide. Am J Clin Pathol 1951; 21: 1153–72.
- 16 Puri CP, Gopalkrishnan K, Iyer KS. Constraints in the development of contraceptives for men. Asian J Androl 2000; 2: 179–90.
- 17 Dennis LK, Dawson DV, Resnick MI. Vasectomy and the risk of prostate cancer: a meta-analysis examining vasectomy status, age at vasectomy, and time since vasectomy. Prostate Cancer Prostatic Dis 2002; 5: 193–203.
- 18 Manivannan B, Mishra PK, Lohiya NK. Ultrastructural changes in the vas deferens of langur monkeys *Presbytis entellus entellus* after vas occlusion with styrene maleic anhydride and after its reversal. Contraception 1999; 59: 137–44.