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NON-INVASIVE REVERSAL OF MALE CONTRACEPTIVE RISUG FROM VAS DEFERENS OF *MACACA MULATTA* MONKEYS

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ABSTRACT

Contraception is the term used for the prevention of pregnancy. There are numerous methods to prevent a pregnancy: The use of hormone medications, contraceptive devices (barriers), and surgery. However, these methods are not all equally reliable. Vasectomy is the easiest form of surgical sterilization, it should be considered as a permanent form of birth control, through vasectomy reversal procedure is difficult. It is expensive and not available in most areas of the world. RISUG (Reversible inhibition of sperm under guidance) is a non-hormonal male contraceptive and it could be reversed easily. In male monkeys pretreated vas occluded with RISUG has been attempted on the level of semen physical parameters, semen biochemistry, and its non-invasive reversal procedure has been assessed through palpation, percutaneous electrical stimulation of the vas deferens, vibratory movement, suprapubic percussion, and rectal digital massage of the vas deferens in all injected monkeys after vas occluded. The result revealed that following non-invasive reversal normospermia obtained after two successive ejaculations in all the animals and did not show any remarkable changes in semen physical and chemical parameters. Thus it shows that the RISUG is effective for male contraceptive as well as reversed through non-invasive procedure and has no any adverse effects.

KEYWORDS

RISUG, Non-Invasive reversal: Monkey, Male Contraceptive, Vasocclusion and Styrene Maleic Anhydride.

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INTRODUCTION

Contraception is critical to health, development, and quality of life. Men have even fewer options for controlling their fertility: vasectomy and condoms. A safe, reliable reversible, method for men would therefore meet a critical need. Indeed, men have begun to demand such a method. A Number of investigations to block the lumen of the vas deferens using different chemicals and solid plugs have been carried out¹⁻³. Intervention at the level of the vas deferens holds the

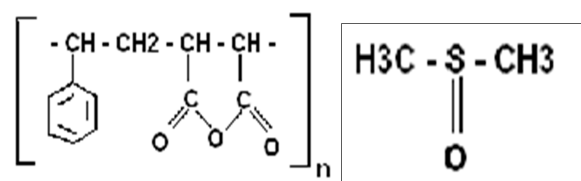
potential of leading to contraceptive techniques with minimal systemic effects. Vasectomy is one of the most widely used methods. Presently vasectomy is the only available surgical male contraceptive that is safe and effective as a one-time procedure. However the acceptance of vasectomy has been confined mostly to a small number of people and its global acceptance rate is only 10%^{4,5}. In vas based contraceptive methods, the status of accessory reproductive glands, especially the seminal vesicle and the prostate, are often being neglected. A limitation of the vasectomy is that its reversibility cannot be possible and therefore need for another method of vas occluded with a higher success rate of reversibility.

Among the several reversible vas-occlusive procedures, Intravasal injectable contraceptive using RISUG is a recent development among vas - based contraceptive, it is an injectable compound that partially blocks the vas deference. This contraceptive has completed phase I and phase II clinical trials^{6,7}, currently it is under phase-III clinical trial⁸. RISUG injected in vas deferens no-scalpel procedure, is considered to be a better alternative to vasectomy⁹. RISUG has well established pH - lowering effects in the internal milieu of the vas and has the positive charges which disturb the negative charge of the plasma membrane of the spermatozoa that pass through the vas lumen^{10,11}. Preliminary investigations following short-term vas occlusion with RISUG in monkeys resulted in oligo astheno teratozoospermia in the initial two or three ejaculations and subsequent azoospermia, suggesting no adverse effects on hematology serum biochemistry and seminal plasma biochemistry. Reversal was done through Non-invasive in different steps was attempted in 5 monkeys. Normospermia was obtained three to four successive ejaculations after non-invasive reversal.

MATERIALS AND METHOD

Drug

RISUG (Reversible Inhibition of Sperm under Guidance) is composed of a copolymer of styrene and maleic anhydride in 1:1 ratio. The Co-Polymer styrene maleic anhydride complex with dimethyl sulphoxide (DMSO) in (1:2) ratio.



(Styrene Maleic Anhydride)

Dimethyl Sulphoxide (DMSO)

Animal

Seven healthy adult male rhesus monkeys of *Macaca mulatta* strain, weighting 9 to 11 kg were used in the study. The animals were housed individually in the Iron cages prior approval from Institutional Animal Ethics Committee (IAEC) of All India Institute of Medical Sciences. Animals were purchased from the Central Animal Facility (CAF), A.I.I.M.S. All the animals were subjected to pretreatment sampling at least 10 day intervals before subjecting them to treatment. The semen samples were collected by electro stimulations and used for physical characteristics and semen biochemistry.

Vas Occlusion

Animals were anesthetized with ketamine hydrochloride (NEON Laboratories Ltd., Mumbai) at 25 mg/kg body wt. A small incision was made close to the external inguinal segment and the vas deferens was exposed from the spermatic cord. 20 μl RISUG was injected into the lumen of the vas After Injection, the spermatic cord sheath was closed with catgut suture. This procedure was performed bilaterally. Post-operative care was provided with antibiotics and anti-inflammatory drugs and all the animals had uneventful postoperative recovered.

Noninvasive reversal

Non-invasive reversal procedure was performed in 5 of 7 vas occluded monkeys under mild anesthesia using ketamine aimed to propel the RISUG from the vas deferens to ejaculatory ducts¹². In this technique involved palpation, percutaneous squeezing of vas deferens at the scrotal and inguinal segment, electrical stimulation and vibration through vibrator at the inguinal segment, supra-pubic percussion and per-rectal digital massage, a finger massage to the ampullary segment of the vas deferens in successive steps. The procedures were repeated 3-4 times.

Parameters

Semen was collected in sterile wide mouth containers through electro-ejaculator using a model 611-stimulator of Phipps and Birds, Inc. using continuous mode on 5-6 events per second, 30-35 millisecond frequency duration and output voltage between 25 and 40 volt after manually restraining animals in their respective cages.

a) Semen Physical parameters

Semen weight, Volume, pH, Color, sperm counts, motility and viability of spermatozoa were recorded as per WHO laboratory manual¹³ for the examination of semen.

b) Seminal Plasma biochemistry

Seminal plasma free of spermatozoa, obtained after centrifugation was used for the biochemical analysis of Acid phosphatase¹³ Fructose¹⁴ and Glycerol phosphoryl Choline (GPC)¹⁵.

RESULTS

There was no any morbidity observed in the animal after non-invasive reversal.

Semen analysis

After non-invasive reversal semen physical parameters: like Volume, Colour, pH and liquefaction time of semen did not show any considerable changes from those of pre-treatment samples.

Non-invasive reversal influence, spermatozoa appeared in the semen with low sperm counts which steadily augment and improved subsequent ejaculations, and normospermia was obtained after third ejaculation. Sperm motility of the animals, from first ejaculation to third ejaculations illustrates gradually improvement following the reversal.

Normal Viability of the spermatozoa were obtained in 4 of 7 animals, partial viability and dead spermatozoa were observed in remaining 2 animals through sperm viability test by Nigrosin-eosin method in first ejaculation. Sperm viability normal like pretreatment was attained subsequent to third ejaculation in all animals (Table No.1).

Seminal plasma Biochemistry

In pre-treated semen samples Acid phosphatase activity was estimated as described WHO manual 52.5 ± 1.5 U/ml, Fructose 12.5 ± 0.5 u/mol, LDH 31 ± 1.05 U/ml, GPC 1.5 ± 0.5 g/ml respectively they slight turn down later than vas occluded (Table No.2). All these parameters illustrated regular improvement following non-invasive reversal and they arrive at pretreatment level after fourth to fifth reversal (Table No.3).

DISCUSSION

Vas occlusion by RISUG is a recent and effective procedure, Styrene maleic anhydride (SMA) is a hydrophilic polymer used in RISUG which origin an initial swelling at the site of administration. The mechanism of action of RISUG may be endorsed in part to a total or partial blockage and also the charge related effect of the polymer, which cause the majority of sperm to disintegrate. A few of those that escapes such an effect were found to be morphologically abnormal and thus they incapable of fertilization¹⁶. Vas occlusion by RISUG resulted in severe oligospermia and azospermia in first two ejaculations and consistent azospermia in the following ejaculations¹⁷.

In the present study attempt with non-invasive reversal was more successful later than vas occluded, pretreatment level of the sperm were expanded after post reversal. The semen biochemistry, i.e the level of Fructose, Acid Phosphatase, Glycerol Phosphoryl Choline (GPC) and Lactate Dehydrogenase (LDH), which are not much inflated by reversal suggesting that this procedure does not affect the reproductive gland. The reduction of LDH and GPC in the seminal plasma in vas occluded animal and their reversal to normalcy, following reversal in the present investigation could be mainly due to obstruction in the vas deferens as these are the components mainly of testicular and epididymal origin¹⁸. However the decline fructose in the seminal plasma could not be related to the functional status of accessory reproductive gland.

Table No.1: Pre-injection

Semen Examination															
a) Macroscopic Examination															
Animal No:-	M-02			M-04			M-05			M-07			M-08		
Sample No.:-	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
1- Colour	White	white	white	white	white	white	white	white	white	White	White	White	White	White	White
2-pH	7.4	7.4	7.4	7.4	7.4	7.6	7.6	7.8	7.6	7.4	7.4	7.2	7.2	7.4	7.4
3- Appearance	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream
4- Total volume	3.1705	2.3387	3.2974	1.9097	1.082	1.028	0.8201	0.7031	1.0884	3.6586	2.8647	2.9153	3.2649	2.9825	2.8932
5- Liquefaction	45 min	45 min	45 min	50 min	50 min	45 min	55 min	50 min	45 min	50 min	50 min	45 min	45 min	50 min	50 min
6- Volume of semen*	1.2948	0.9088	1.3754	0.2786	0.3892	0.298	0.2337	0.2078	0.3542	1.2151	1.0404	1.1319	1.0489	1.0611	1.0011
7- wt. of coagulums	1.8757	1.4299	1.922	1.6311	0.6928	0.729	0.5864	0.4953	0.7342	2.4435	1.8243	1.7834	2.216	1.9214	1.8922
b) Microscopic Examination															
1-Spermmili/ml**	325	290	296	352	348	380	258	270	282	395	420	295	640	345	325
2-Sperm /ejaculat	420.81	263.55	407.11	98.067	135.44	113.4	60.29	56.1	99.88	479.96	436.96	333.91	671.29	366.07	325.35
3- Sperm Motility	85	80	80	80	70	85	80	85	85	80	70	80	70	75	70
a- Rapid progressive %	45	40	45	40	25	35	40	40	40	40	30	50	40	35	35
b-Sluggish progressive %	30	25	15	20	20	20	30	30	30	20	30	15	20	25	20
c- Non progressive %	10	15	20	20	25	30	10	15	15	20	10	15	10	15	15
d- Immotile %	15	20	20	20	30	15	20	15	15	20	30	20	30	25	30
4- Sperm Viability %	90	90	85	85	75	90	85	90	90	95	88	95	94	88	90
5- Sperm Morphology	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
c) Seminal Plasma Biochemistry Examination															
1- Fructose	13	12.5	12.8	11	12.25	11.54	11	11.2	12.25	11.5	10.9	11.2	11	12.2	11.5
2- Acid phosphatase	54.52	53.25	52.85	50.2	50.75	49.8	49	51.25	50.8	52.5	49	53.5	49.2	48	48.2
3- LDH	27.52	26.8	27	25.5	24.2	24.5	22.25	24	23.5	24.9	25.5	25	24	22.8	23.5
4- GPC	2	1.9	2	1.62	1.55	1.7	1.65	1.72	1.6	1.8	1.95	2.1	1.9	1.8	1.9

*Volume = in ml ; **Sperms count in million per millilitre, A = Abnormal sperms: N = Normal, Occ = Occasional, C = Curve

Table No.2: Post-injection

Semen Examination															
a) Macroscopic Examination															
Animal No:-	M-02			M-04			M-05			M-07			M-08		
Sample No.:-	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
1- Colour	white	white	white	White	White	White	White	White	White	White	White	White	White	White	White
2-pH	7.4	7.2	7.5	7.2	7.2	7.4	7.4	7.4	7.2	7.4	7.2	7.2	7.4	7.4	7.4
3- Appearance	cream	cream	cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream
4- Total volume	0.0505	3.171	3.297	0.8566	1.2236	1.625	0.6366	3.569	1.126	0.925	1.297	1.233	0.9627	0.975	1.2105
5- Liquefaction	45 min	45min	45min	50 min	50 min	45 min	50 min	45 min	45 min	45 min	50 min	50 min	50 min	50 min	50 min
6-Volume of semen*	0.0162	1.295	1.375	0.2543	0.3452	0.443	0.291	1.0186	0.271	0.271	0.374	0.32	0.2073	0.293	0.2891
7- wt. of coagulums	0.0343	1.757	1.922	0.6023	0.8784	1.183	0.345	2.5504	0.855	0.654	0.923	0.913	0.7554	0.682	0.9214
b) Microscopic Examination															
1-Sperm mili/ml**	2	Occ	0	3	Occ	Occ	3	Occ	Occ	5	Occ	Occ	10	Occ	Occ
2-Sperm /ejaculat	0.324	0	0	0.7629	0	0	0.873	0	0	1.355	0	0	2.073	0	0
3- Sperm Motility	33	0	0	15	0	0	3	0	0	10	0	0	5	0	0
a- Rapid progressive%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
b-Sluggish progressive%	5	0	0	3	0	0	0	0	0	5	0	0	0	0	0
c- Non progressive%	28	0	0	12	0	0	3	0	0	5	0	0	5	0	0
d- Immotile%	67	0	0	85	0	0	97	0	0	90	0	0	95	0	0
4- Sperm Viability%	55	0	0	55	0	0	40	0	0	50	0	0	45	0	0
5- Sperm Morphology	A	C	0	A	C	0	A	C	0	A	C	0	A	C	0
c) Seminal Plasma Biochemistry Examination															
1- Fructose	9.5	11	10.25	10.5	10	9.5	11.8	10.5	10	11.2	11	11.5	11.2	10.2	9.5
2- Acid phosphatase	38	41	38.75	40.5	38	37	39	37	37	40	39	38.5	39.5	39	38
3- LDH	22	24	22.8	20	18	17	18.5	18	17.5	21.2	19.5	18	18	17.5	17
4- GPC	0.5	0.39	0.4	0.4	0.45	0.5	0.45	0.48	0.35	0.5	0.38	0.42	0.4	0.38	0.32

Table No.3: Post-reversal

Semen Examination															
a) Macroscopic Examination															
Animal No:-	M-02			M-04			M-05			M-07			M-08		
Sample No.:-	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
1- Colour	white	white	white	white	white	white	white	white	---	white	white	white	white	white	white
2- Ph	7.2	7.2	7.5	7.2	7.4	7.4	7.4	7.4	---	7.2	7.2	7.4	7.4	7.2	7.4
3- Appearance	cream	cream	cream	cream	cream	cream	cream	cream	---	cream	cream	cream	cream	cream	cream
4- Total volume	1.5232	2.8025	3.2302	0.8652	0.7624	0.983	3.0254	2.986	---	3.0258	2.1526	2.6232	2.8243	2.6072	2.5681
5- Liquefaction	50 min	45 min	45 min	45min	50min	50min	50 min	45min	---	45 min	50 min	45 min	50 min	45 min	45 min
6-Volume of semen*	0.4652	0.8642	1.3105	0.2022	0.2025	0.283	1.0225	0.856	---	1.008	0.8253	0.9024	1.0232	0.8062	0.8256
7- wt. of coagulums	1.058	1.9383	1.9197	0.663	0.5599	0.699	2.0029	2.129		2.0178	1.3273	1.7239	1.8011	1.801	1.7425
b) Microscopic Examination															
1-Sperm mili/ml**	25	95.5	150.5	18.8	86.2	130	29	100.5	---	22	98	135	25	95.5	145
2-Sperm /ejaculat	11.63	82.53	197.23	3.801	17.455	36.81	29.65	86.07	---	22.176	80.879	121.82	25.58	76.99	119.71

3- Sperm Motility	45	50	75	20	40	70	25	50	---	30	40	40	30	40	50
a- Rapid progressive%	5	10	35	4	5	20	5	10	---	5	10	10	10	10	15
b-Sluggish progressive%	15	10	15	6	10	25	5	15	---	10	10	15	5	10	15
c- Non progressive%	25	30	25	10	25	25	15	25	---	15	20	15	15	20	20
d- Immotile%	55	50	25	80	60	30	75	50	---	70	60	60	70	60	50
4- Sperm Viability%	65	60	95	50	65	85	55	65	---	45	55	50	40	45	55
5- Sperm Morphology	coil + N	N	N	coil+ N	N	N	Coil+N	N	---	Coil+N	N	N	Coil+N	N	N
c) Seminal Plasma Biochemistry Examination															
1- Fructose	11	12.5	13.5	10	11.5	12.5	9.5	11	---	10.9	11	11.7	10.8	11.2	11.5
2- Acid phosphatase	52.1	53.5	54	48.1	49.8	51.1	49.5	50.3	---	48.7	49.2	49.5	48.8	49	49.2
3- LDH	24	26	26.5	22.1	23.3	24.8	22.5	23.5	---	22.5	22.7	23.7	21.5	21.8	23.5
4- GPC	1.6	1.8	1.9	1.5	1.6	1.8	1.4	1.6	---	1.3	1.5	1.9	1.2	1.5	1.7

CONCLUSION

The result of the present investigation expresses that the reversal of vas occluded by RISUG through a non-invasive approach is feasible even after reversal and restoration to normalcy of morphological and physical characteristics of spermatozoa following vas occlusion and reversal makes RISUG globally more adequate than any other vas based method.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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