

“Histo-architecture of the rabbit fallopian tube following short-term RISUG implant”.

Mithilesh Pal¹; Pankaj Kumar Bhatia²; Bhardwaj, J.C¹

¹Dept. of Biomedical Engineering, All India Institute of Medical Sciences, New Delhi
& Indian Institute of Technology, Delhi

²Dept. of Neurology All India Institute of Medical Sciences, New Delhi
Email- pal.mithilesh@gmail.com

Abstract:

RISUG ® (Reversible Inhibition of Sperm Under Guidance) is an injectable contraceptive for male running under clinical trial phase- III. As RISUG was not tried in females, the present study was initiated to examine the tissue specific reaction and the histo-architecture of the fallopian tube, isthmus region, receive the polymer implant. Five non-pregnant female rabbits were allowed to retain the implant for 90 days. Thereafter, isthmuses along with other associated organs were removed and examined both macro- and micro-scopically. There were no gross changes in the isthmus region and other related organs like ampulla, uterus and ovary. Aggregates of RISUG ® implants were observed occupying spaces among villi, bulging with shed epithelium and mucus in the lumen, villi deviated from their normal perpendicular position. No lymphocytic infiltration in mucosa and tunica muscle layer was observed. The serosa showed normal histo-architecture in the implanted and adjoining isthmus wall. The above finding indicates that the drug is compatible with in the fallopian tube and therefore needs to be explored further for its contraceptive potential in females.

Key words: Contraceptive, Fallopian tube, Isthmus, RISUG ®, Tubal implant,

Introduction:

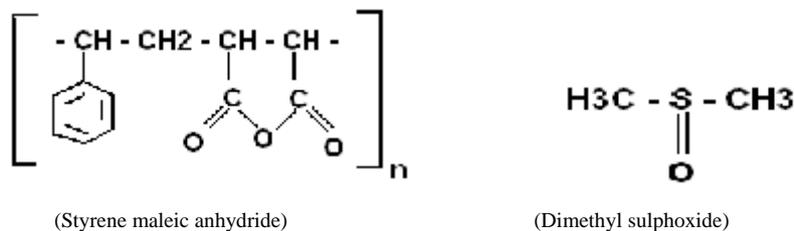
Among available female contraceptives and sterilization techniques, each one has its own limitations and side effects, whether they are short term or long term, of hormonal or non hormonal tubal sterilization.

The focus of present study was on non-hormonal specialized polymer-SMA¹ (Styrene Maleic Anhydride) dissolved in Dimethyl sulphoxide (DMSO) form RISUG ®². Drug was injected as an implant into the lumen of isthmus to observe the morphology of fallopian tube and related organs, also the histoarchitecture of the isthmus region surrounding implant in the lumen and adjoining areas. The present study was designed to develop a female contraceptive.

Several research reports on light and electron microscopic structure of rabbit oviduct^{3,4,5}, reviews^{6,7} and chapters^{8,9,10}, are available on the histology and cytology of the different parts of the tube in estrus, nonestrus phase and their characteristics secretions in different parts associated with specialized cellular organization.

Material & Methods:

Five mature New Zealand white parous, fertile female rabbits weighing 2.0 to 2.5 kg were used for the study. A prior approval from Institutional Animal Ethics Committee (IAEC) of All India Institute of Medical Sciences (AIIMS) was obtained in the year 2012. Animals were purchased from Central Animal Facility (CAF), A.I.I.M.S., and were kept at 12 hrs. light and dark cycle, on normal animal food diet and water ad-libitum. RISUG is composed of styrene maleic anhydride (SMA) copolymer complex with the solvent dimethyl sulphoxide (DMSO) in a ratio of 1:2 (Weight-mg: volume-microlitre).



Rabbits were weighed and ventral abdominal skin was shaved with anti-septic solution. Dicrystecine (Zydus Animal Health Ltd., Amdabad) injection 0.5 ml was given intra muscular (IM) before anesthesia. Intra muscular injection of Ketamine hydrochloride (NEON Laboratories Ltd., Mumbai) 25mg/kg body wt. was injected and after 5-7 minutes skin was press tested by toothed forceps to judge the conscious level of the animal. After reaching the surgical anesthetic condition, a ventro- median incision between the last teats was made with scalpel blade. Uterus was located and utero- tubal junction was identified to inject the RISUG ® in the lumen of the isthmus. The isthmus was picked up with looped forceps, mesenteries were retracted at the site

of injection, scalpel handle was used as back support for isthmus, a 15mm length of 23G needle with 40° bevel angle tip was inserted in the lumen of fallopian tube at 35° – 40° angle with fallopian tube, pointing the needle towards ampullary junction to avoid inadvertent puncture.

Ten micro liter RISUG[®] was injected bilaterally in three animals. In two animals' only right isthmus were injected RISUG[®] and contralateral tubes were injected 10 µl normal sterile saline that served as control. The incised skin and muscles were sutured with topical application of antiseptic powder. Post-operatively rabbits received antibiotics for three days. Animals were observed daily and weighed weekly to check weight loss or gain if any. After 90 days, animals were sacrificed to obtain implanted and non-implanted isthmuses, along with other related anatomical structures like ampulla, ovary and uterus of both sides. Macroscopic examination was done on implanted site and other adjoining organs and photographed. These organs were trimmed and processed for histochemistry and were partly fixed immediately in 10% buffered neutral formalin for about 24 hours at 4°C in an upright position in pre-numbered Petri-dishes.

Next day organs were trimmed under magnifying lens and were numbered, pieces of tissues were passed through ascending series of alcohol and embedded in paraffin wax. Serial sections were cut at 7 µM thickness and stained with haematoxylin and eosin stain to study the cellular details.

Results:

Macroscopic findings: The implanted isthmuses did not show any sign of dilation, swelling, contraction, scarring or abnormal growth in wall, no adhesions with other abdominal organs after 90 days. Normal spiral/convoluted structure of oviducts with ovary and uterus were observed, an erythematous spot was noted in one of the implanted isthmuses.

Microscopic findings: Longitudinal sections (L.S.) of implanted isthmuses showed denudation of epithelium and moderately blunt villi with aggregates of implant material in the lumen, axial portion of prominent villi was partially flattened due to compression, even some villi showed bifurcation shaped as a scavenge appearance, inner circular and outer longitudinal muscles were normal, few giant cells with foreign material in muscular layer along with epithelial attenuation were observed (Fig- 1).

Some transverse sections (T.S.) showed shed epithelium and mucus bulging with implanted material aggregates but lumen remained patent (Fig – 2). In some tissue sections compression and mild curving of villi and detached epithelial cells with mucus and implanted material in lumen were noted (Fig -3 & Fig -4).

In one of the implanted isthmus, serosa showed presence of lymphocytes and macrophage infiltration. Giant cells with foreign material (Fig -5) and mild fibrosis was found in the serosa in one of the 8 isthmuses implanted.

The transverse sections of adjoining implanted site, isthmus showed prominent tunica serosa, thick layer of inner circular and outer longitudinal muscle fibers. The lamina propria was well marked and mucosal folds were thrown in lumen in the form of conical villi; number and size of villi were always less in isthmus as compared to ampulla. Four or five mucosal folds were more prominent in size and protruding deep in lumen (Fig-6). In the base of larger villi, very prominent lamina propria along with well demarcated blood vessels and connective tissues in the lamina were observed. Dominant ciliated cells showed fairly clear cytoplasm. The epithelium contained secretory cells in trough had an intensely fine granular cytoplasm, and a club-shaped apical end protruding into the lumen (Fig- 7).

Transverse sections of control isthmus showed villi and muscularis normal with lumen patent, lamina propria with blood vessels and connective tissues, epithelial layer of villi normal (Fig- 8).

Discussion:

Oviduct is intimately associated with the physiological processes of its timely occupants: the secretory fluid, fertilized or unfertilized ovum, and the spermatozoa. Their transportation is certainly important for successful completion of reproductive process. Any interference in the transportation may result in the failure of fertilization¹¹. In the present investigation this only "Failure of Fertilization" concept has been explored as contraceptive method without any pathology and an implant may serve the purpose of contraception.

A previous study on tubal implant with polyethylene tubing to bypass tubal passage of gamete or zygote from ampulla to uterus reported no conception after mating¹². Another study was on tubal occlusion with 'Silastic beads' implant to observe the fertility¹³. Other two studies on ligation of tubes to obstruct the passage of gametes^{14, 15} were carried out to check fertility and feasibility of reversibility by reanastomosis^{14, 16}. The objective of previous studies was to block the passage of gametes in the lumen, either in the ampulla or in the isthmus region with implants. They did not study the physiology, biochemistry and histoarchitecture of the tubes after implant, but, only fertility outcome was checked after mating.

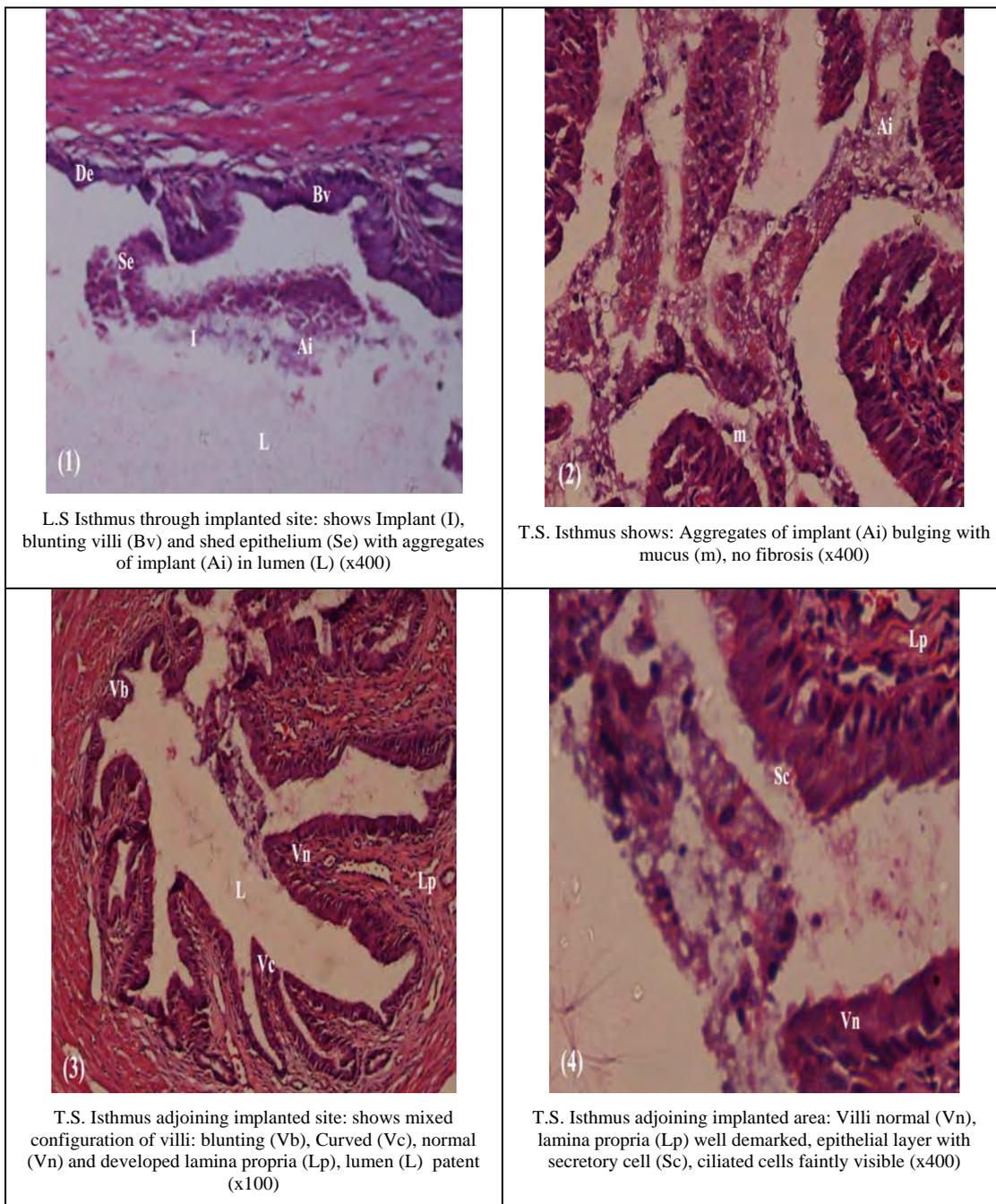
The present study aims to investigate histoarchitecture of the tubes after short term retention of implant. The effects of RISUG[®] implant in the lumen of 8 isthmus from 5 rabbits after three months were observed for

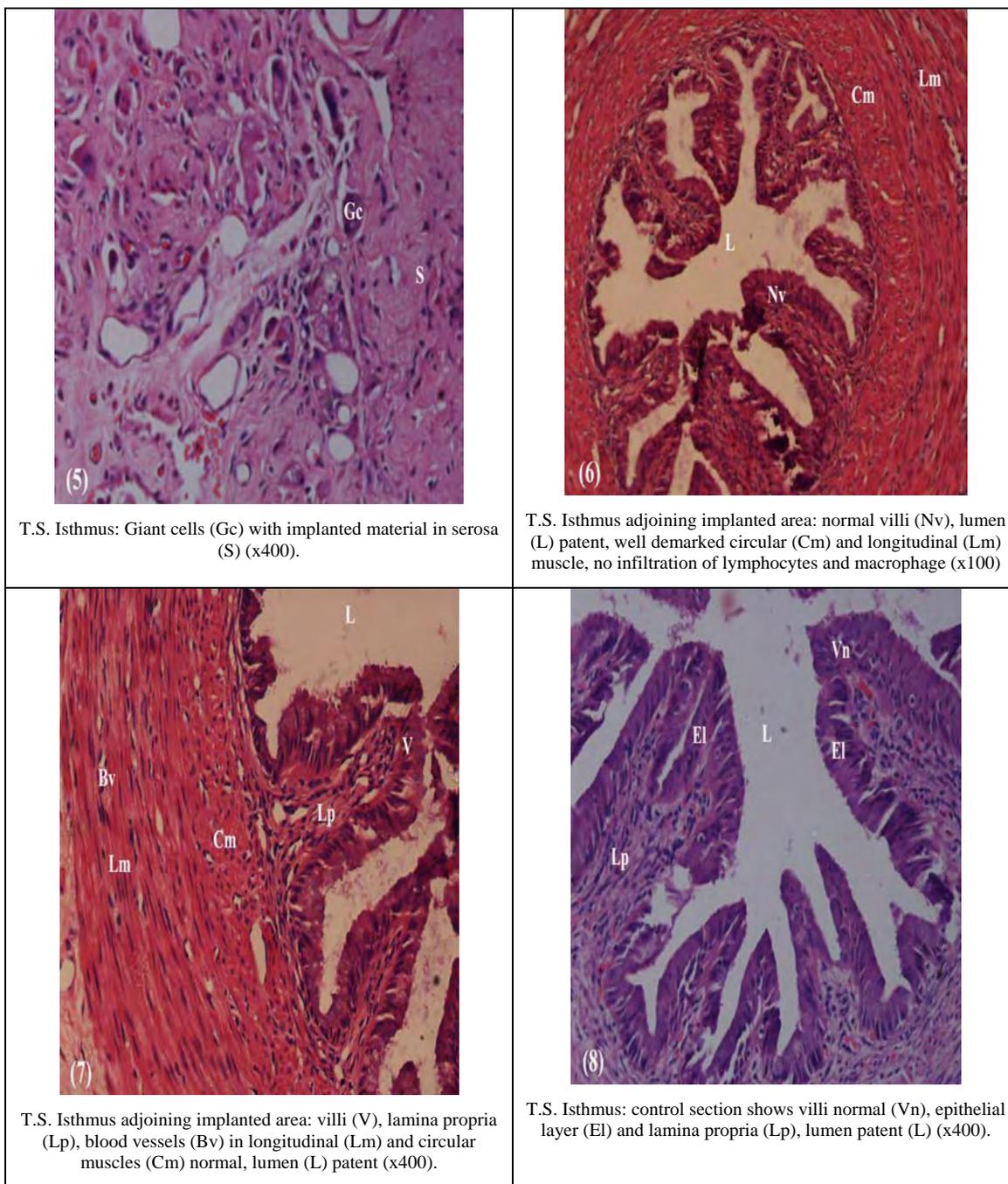
the anatomy of organs and histology of oviductal tissues; morphology of epithelium, mucosa, villi, patency of lumen and other tissue reaction within the mucosa and the serosa of the isthmuses.

Microscopic examination of all implanted isthmus with RISUG[®] gel showed alterations within the area of injected lumen and minimum alterations in adjoining areas. There was not much variation in epithelial lining and muscular arrangement, lumen was patent. Only in one implanted isthmus mild fibrosis was observed, there was no mucosal fibrosis in any specimen.

Conclusion:

After short term retention of RISUG[®] implant in lumen of isthmus did not show infiltration of lymphocytes and macrophage in majority of tissues, adjoining isthmus showed normal epithelium and serosa comparable to control except in one tissue. There was no pathology in mucosa and muscular layer. Implant did not show any tissue specific reaction around injected site.





References:

- [1] Misro MM, Kaur H, Mahajan S, Guha SK. An Intravasal non-occlusive contraceptive device in rats. *J.Reprod Fertil.* 1982; 65(1):9-13
- [2] Guha SK, Singh G, Srivastav A, Das HC, Bhardwaj JC, Mathur V, et al. Two years clinical efficacy trial with dose variations of a vas deferens injectable contraceptive for the male. *Contraception* 1998; 58:165-174.
- [3] Nilsson, O. Electron Microscopy of the Fallopian tube Epithelium of Rabbits in Oestrus. *Exp.Cell Res.*1958; 14:341-354.
- [4] Nilsson O and Reinius S. Light and Electron Microscopic structure of the oviduct In: Hafez ESE, Blandau RG, editors. *The Mammalian Oviduct* Chicago III University of Chicago Press; 1968 p. 57-83.
- [5] Pauerstein CJ, Alexander RW, Mobley JA, Fremming BD. Comparative anatomy of the inner longitudinal muscle layer of oviductal isthmus. *Obstet.Gynecol.* 1970; 35(4):504-512.
- [6] Jansen RP. Ultrastructure and histochemistry of Acid mucus glycoproteins in the estrus mammal oviduct. *Microsc. Res. Tech.*1995; 32 (1): 24 – 49 (Review).
- [7] Muglia U, Motta PM. A new morpho-functional classification of fallopian tube based on its three dimensional myoarchitecture. *Histo. Histopathol.* 2001; 16(1):227-237(Review).
- [8] Hafez ESE and Black DL .The mammalian Uterotubal junction. In: ESE Hafez, RJ Blandau editors. *The Mammalian oviduct.* Chicago (USA); The University of Chicago Press; 1969.p. 85 to 126.
- [9] Beck LR and Boots LR. *The Comparative Anatomy, Histology and Morphology of the Mammalian Oviduct.*, In: Beck LR, Boots LR, editors. *The Oviduct and its function.* New York & London; Academic Press, Inc.1974, p 1 to 51.
- [10] James E wheeler MD. Disease of fallopian tube.Chapter-14, In: Robert j Kurman, editor. *Blaustein's Pathology of the Female Genital Tract IV-Edition.* New York, USA Springer-Verlag,. 1994, p. 529 – 561.

- [11] Mastroinni Luigi Jr. Potentials for fertility regulation at the tubal level. *Contraception* 1976; 13 (4): 447.
- [12] Mrouch A. Effect of Tubal Implantation on rabbit Fertility. *Fertil. Steril*; 1969;20(6):928 – 932.
- [13] Heesch CM and Hodgson BJ. Medicated intratubal silastic spheres do not prevent pregnancy in rabbits. *Contraception* 1976; 13 (4): 497 – 501.
- [14] D.Bernhardt – Huth;Ch Frantzen and H.W. Schlosser . Morphological Alterations of Rabbit oviducts Following Ligation of the Tubal Isthmus. *Virchows Arch. A Path. Anat. and Histol.* 1979; 384:195-211.
- [15] D.Bernhardt – Huth; H.W. Schlosser and Ch Frantzen . Morphological Alterations of Rabbit oviducts after Ligation of the Isthmus or Ampulla *Arch Gynecol* 1980; 229: 167 – 176.
- [16] D.Bernhardt – Huth; Frantzen C and Schlosser H W. Morphology of Rabbit oviducts after Microsurgical Techniques for reanastomosis of the Isthmus or Ampulla. *Arch. Gynecol.* 1981; 230(3): 251 – 262