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ANTI IMPLANTATION, ANTI OVULATORY AND ANTI ESTROGENIC STUDIES OF THE AQEUOUS EXTRACT OF FICUS INFECTORIA (PILKHAN) IN RODENTS

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ABSTRACT

Screening of aqueous extract of *Ficus infectoria*, on the following were assessed: Anti implantation, Anti ovulatory in *wistar albino* rats and Anti estrogenic in immature *swiss albino* mice. Aqueous extract of *Ficus infectoria* (100 mg/kg) was administered by gavage daily from day 1-7 after getting clump of spermatozoa in their vaginal smear for Anti implantation activity. On the 10th day of pregnancy laparotomy was performed under general anesthesia. Count the number of implants from the uteri and corpora lutea from the ovary. Calculate the Pre-implantation loss and Post-implantation loss by the equation. Aqueous extract of *Ficus infectoria* (100 mg/kg) was administered by oral route from day 1-3 for Anti ovulatory activity. Figured out the anti ovulatory activity by the comparison of histopathology of ovary to the control group. Aqueous extract of *Ficus infectoria* (100 mg/kg) was administered by oral route daily from day 1-4 in immature *swiss albino* mice for anti estrogenic activity. It was deciphered by the comparison of the weight of uteri to control and estrogen administered group. Drug possessed increased post implantation loss then the pre implantation loss. Drug exerted the significant loss of corpora lutea in the histopathology. Drug did not possess the uterotropic effect on the uterus by significant weight loss.

KEYWORDS: Aqueous extract of *Ficus infectoria*, Anti implantation, Anti ovulatory, Anti estrogenic.

1. INTRODUCTION

Hormonal contraceptives cannot be used for long duration due to their severe effects.[1]

Available local contraceptive measures do not give 100% surety of prevention of conception.[2] Permanent measures are surgical therapies only. Hence, there is a need to evaluate alternate and safe formulations from indigenous systems of medicine for temporary as well as permanent sterilization.[3] *Ficus infectoria* (Syn. *Ficus virens*, *Ficus lacor*) is a plant belonging to the genous ficus and family *Moraceae* is found in India, Southeast Asia and Northern Australia. Its common name is white fig and its locally in Hindi language known as *Pilkhan*.[4] It has been reported that all parts of plant are use full in diseases of blood, uterus, burning sensation, hallucinations and unconsciousness, and also *in vitro* antioxidant property.[5] In the ancient literature has been reported that this drug majorly used to treat the uterine disorders and specially in leucorrhoea.[6] There for the present study was aimed to find the evidence in to the rodents by administered the aqueous extract of *Ficus infectoria*.

2. MATERIALS AND METHODS

2.1 Animal approval

Institutional animal ethical committee (IAEC) of the Department of Pharmacology, DELHI PHARMACEUTICAL SCIENCES AND RESERCH UNIVERSITIES, INDIA has approved the study for the use of albino rats (*RATTUS NORVEGICUS*) and albino mice (*MUS MUSCULUS*) of *wistar* and *swiss* strain respectively. (Protocol No. IAEC/2017-I/Prot.No.R-07 dated 1-09-2017). The animals were stored in Animal house of DPSRU. All the animals were housed under the identical conditions. The room temperature was maintained at 25 ± 1˚c throughout the study. The animals were fed food and water *ad libitum* and a balanced diet was maintained in the form of "Golden Feed".

2.2 Anti implantation activity

Adult female rats weighing 140-180 g and in proestrous phase were kept with adult male rats. The following morning, the females were examined for evidence of copulation and those showing thick clumps of spermatozoa in their vaginal smears were separated for the experiment. And the spermatozoa was detected as consider as day 1 of pregnancy. 12 albino rats were divided in to 2 groups of 6 animals. Group 1 served as control and received distilled water. Group 2 received aqueous extract of *Ficus infectoria* at dose of 100mg/kg body weight orally on day 1-7 post coital with the help of catheter. On day 10 of pregnancy, the animal was laparotomized and the number of implants present in both the uterine horns as well as the number of corpora lutea on each ovary was counted. The animals were allowed to complete

the gestation period (usually 21 – 23 days) and the number of litters delivered, if any were counted. The animal was observed for another 10-12 days and number of litters delivered (if any) were counted.[7] By comparing the numbers of implants with the number of litters delivered, the anti implantation activity was calculated. Pre implantation loss and post implantation loss were calculated using following formulas.[8]

1) % Pre implantation loss:

$$\frac{No.\,of\ C.L. - No.\,of\ implants}{No.\,of\ C.L.} \times 100$$

C.L. = Corpus lutea

2) % Post implantation loss:

$$\frac{No.\,of\ implants\ - No.\,of\ litters}{No.\,of\ implants} \times 100$$

2.3 Anti ovulatory activity

Ficus infectoria was screened for it's possible anti ovulatory activity in albino female rats by examining whether they inhibited cupric acetate – induced ovulation. Animals were kept in isolation for at least 21 days to ensure that they were no pregnant and to prevent the induction of ovulation by mating. After, isolation these animals were divided into 2 groups of six animals each. The following extract was administered from day 1st to 3rd by oral route. *Ficus infectoria* was administered at dose level of 100 mg/kg body weight in Group 2 animals. The Group 1 severed as control group and received distilled water by oral route. Thirty minutes after the administration of the last dose, a freshly prepared 0.4% solution of Cupric acetate was administered to each animal intravenously at dose of 4 mg/kg body weight to induce ovulation. To observe ovulation, all rats were sacrificed and the ovaries examined after the18-24 h of the administration of Cupric acetate. Then the ovaries were excised and the tissues were stored in 10% buffered formalin (37-40% formaldehyde in 100ml) sodium phosphate monobasic 4g, sodium phosphate diabasic 6.5 g, distilled water 900ml) solution and subjected to histopathological evaluation.[9]

2.4 Anti estrogenic activity

For the anti estrogenic activity was executed on immature female swiss albino mice, about 3 weeks old weighing approximately 8-10 grams. Animals divided into 4 groups of 5 animals each and dosed according to the following protocol.

Group – 1: 2 ml Distilled water (Control)

Group - 2: 0.5 µg/ml Estradiol benzoate in olive oil (Standard group)

Group - 3: 100 mg/kg body weight aqueous extract of *Ficus infectoria* (Orally)

Group - 4: 100 mg/kg body weight aqueous extract of *Ficus infectoria* (Orally) followed by 0.5 µg/ml of Beta Estradiol in olive oil (Sub cutaneously)

The treatment was continued for 4 days. 24 hours after the last dose, the animals were sacrificed and their uteri dissected, pressed and weighed. The weight of uteri was recorded. Mean value of each group was calculated and expressed as percent reduction of uterine weight compared to controls treated with estradiol alone. Significance (if any) of the animals treated with the drug when compared with those of the animals in the control group was determined.[10]

2.5 Statistical analysis

One way analysis of variant (ANOVA) followed by Tukey's test was used to analyse the effect of aqueous extract of *Ficus infectoria* when compared to control; $P<0.05$ was considered significant.

RESULT

3.1 For anti implantation activity

Aqueous extracts exhibited a mean pre implantation loss was found to be 65.78 ± 2.31% as compared to control group. The mean post implantation loss of *Ficus infectoria* was found to be 100 ± 0.0% as compared to control group. The control group that received distilled water showed a mean pre implantation loss and post implantation loss was found to be 27.507 ± 3.62 and 13.43 ± 3.0 %.

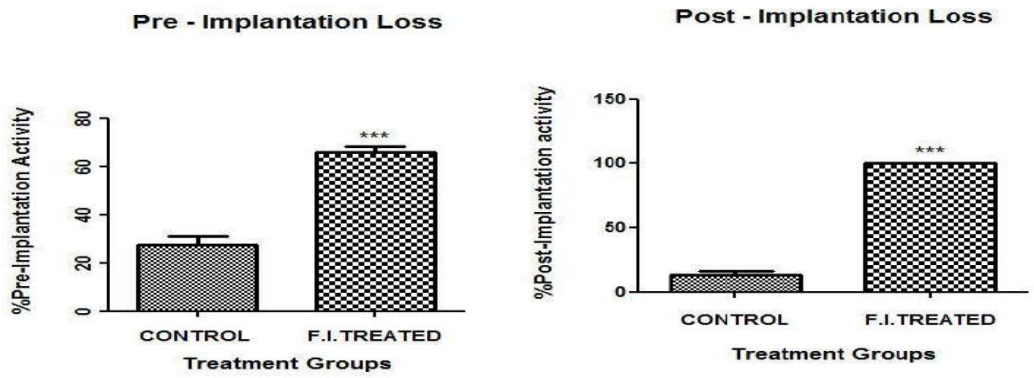

Fig. 1: Pre implantation loss and Mean Post implantation loss in *Ficus infectoria* (100 mg/kg) treated group. ***P < 0.05, Comparison made with control group.

3.2 For anti ovulatory activity

Group 1 histopath shows the ovarian tissue covered by a single layer of modified mesothelium representing the surface epithelium. The ovarian stroma was indistinctly divided into a cortical and a medullary region. The stroma was composed mainly of spindle-shaped stromal cells resembling fibroblasts, typically arranged in an (irregularly) whorled pattern. Ovarian follicles in different stages of maturation (i.e., primordial, maturing [primary, secondary, tertiary and Graafian], and atretic), together with *corpora lutea* and corpora albicantia were present. The primordial follicles contained germ cells only while the maturing follicle was composed of the oocyte, the granulosa layer, and the theca layers. Ovaries from the control group also showed large haemorrhagic corpora lutea with fresh blood filled cavities within the body of the corpus luteum. Mature corpora lutea, 1.5 to 2.5 cm round yellow structures with lobulated outlines and a cystic centre were also seen. Both the granulosa and the theca cells showed prominent luteinisation (Fig: 2 G-1 Control). Group 2 histopath shows numerous follicles in the earlier stages of maturation; the number of *corpora lutea* seen in this group was noticeably less than that seen in the control group. Haemorrhagic *corpora lutea* has not seen in specimens of the mature ovary examined in this group. (Fig: 2 G-2 F.I. treeated)

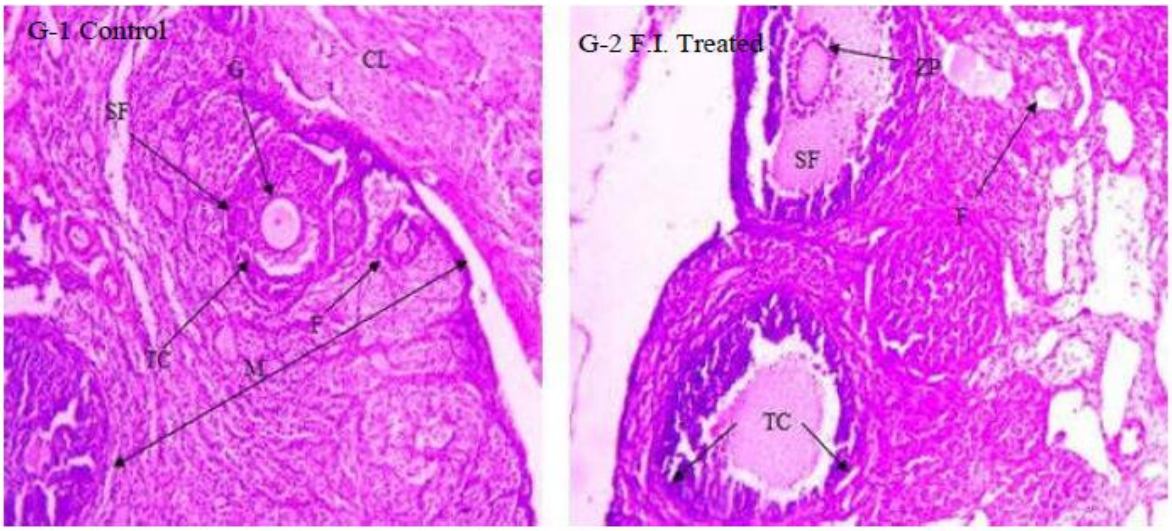

Fig. 2: G-1 control group histopath shows corpus luteum along with its luteinized cells and primary follicle. G-2 *Ficus infectoria* treated group histopath shows mature ovary contains numerous primary follicles with theca interna cells lining the follicular cavity. CL- Corpus luteum, SF- Secondary follicle, F- Primary follicle, M- Medulla, TC- Theca interna cell, G-Granulosa cells, ZP- Zona pellucid.

3.3 For anti estrogenic activity

Its anti estrogenic effect of aqueous extract could be explained with the help of a mean weight of uterus. *Ficus infectoria* does not possess any uterotropic effect on uterus that exhibited an anti estrogenic activity (Group 3). The mean anti estrogenic activity of *Ficus infectoria* was found to be 17.04 ± 0.92 as compared to control group. Aqueous extract of *Ficus infectoria* at dose of 100mg/kg body weight was administered followed by 0.5 µg beta-estradiol administered subcutaneously for day 1st to 4th in immature female mice. Its anti estrogenic effect could be explained with the help of a mean weight of uterus. *Ficus infectoria* induced the uterotropic effect on uterus which possessed by beta-estradiol that exhibited an anti estrogenic activity (Group 4). The mean anti estrogenic activity of *Ficus infectoria* was found to be 31 ± 0.92 as compared to standard group. The control group received distilled water shown a mean weight of uterus was found to be 25 ± 1. The standard group received 0.5 µg beta-estradiol which shown a mean weight of uterus was found to be 54.6 ± 1.07.

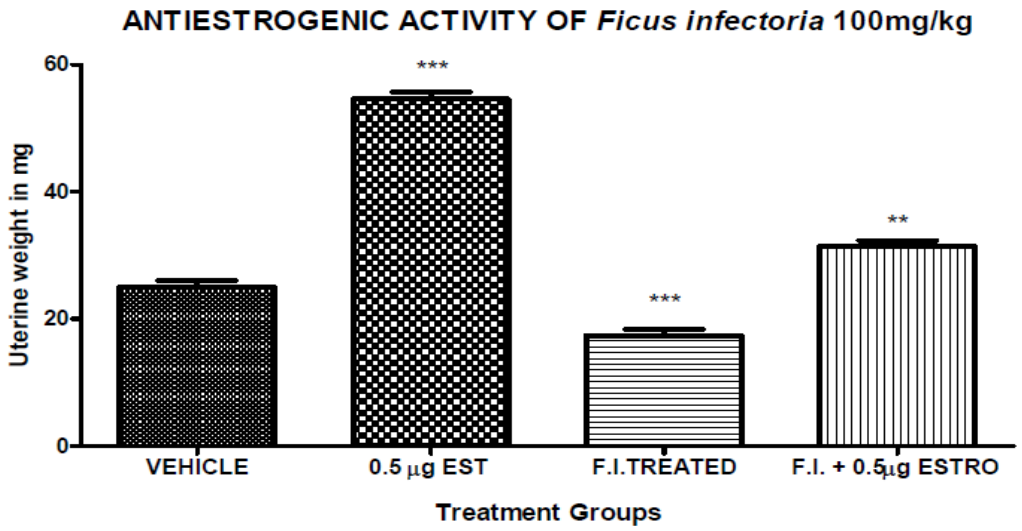

Fig. 3: Mean weight of uterine in control group, 0.5 µg *estradiol* treated group, *Ficus infectoria* (100 mg/kg) treated group and *Ficus infectoria* (100 mg/kg) followed by 0.5 µg *estradiol* treated group. ***P < 0.05, Comparison made with control group. **P<0.05, Comparison made with 0.5 µg *estradiol* treated group.

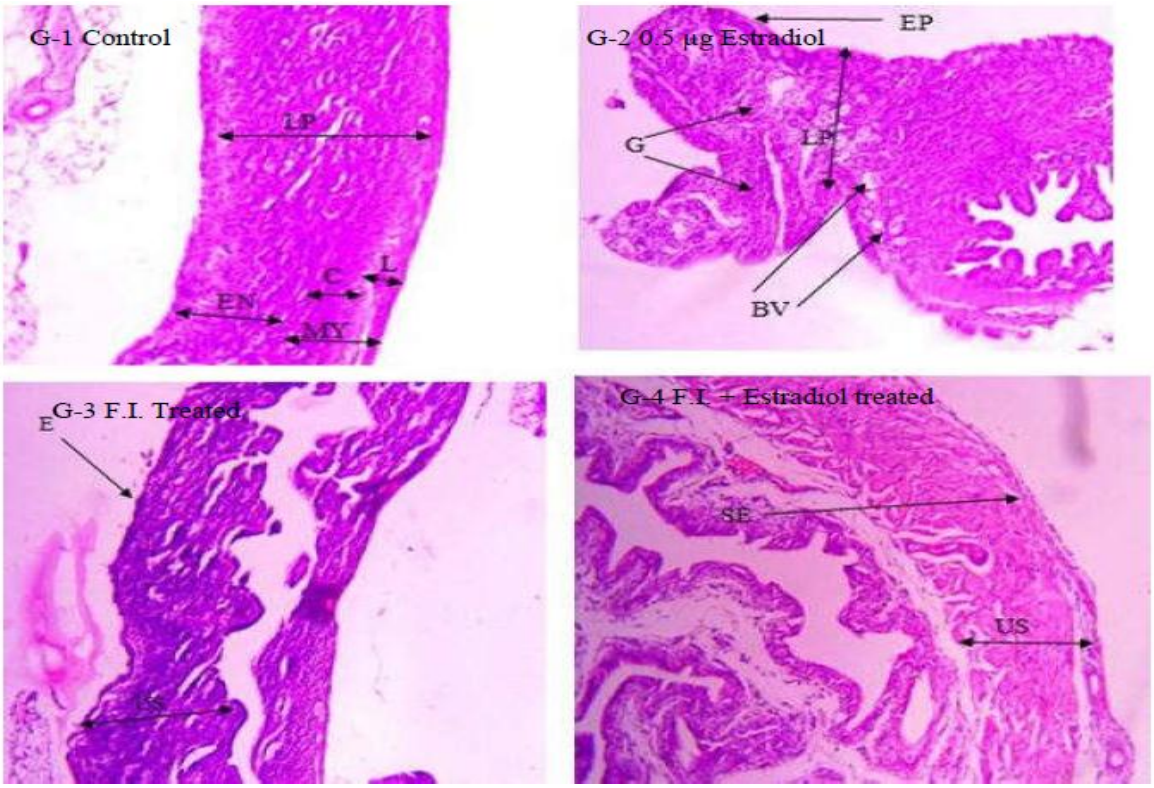

Fig. 4: G-1 control group histopath shows in immature uteri contains flat lining and undifferentiated endothelium, low epithelial layer and undifferentiated stromal layer. G-2 0.5 µg estradiol treated group histopath shows hyperplastic lining thrown into folds. Glandular differentiation in stroma and endometrium and columnar changes in the epithelial lining. G-3 *Ficus infectoria* treated group histopath shows proliferate phase showing epithelial lining and undifferentiated stroma. G-4 *Ficus infectoria* followed by 0.5 µg estradiol treated group histopath some features of differentiated both in the uterine lining and stroma.

LP- Lamina propria, C- Circular layer, L-Longitudinal layer, EN- Endometrium layer, My- Myometrium layer, EP- Surface endothelium, G- Immature endothelial gland, BV- Blood vessels, UN- Undifferentiated stroma, E- Epithelium, SE- Surface epithelium.

DISCUSSION

Anti-implantation activity observed successfully for this the drug. There should be synchronization between development of egg and the endometrium. This is under the influence of a delicate balance and interaction of estrogen and progesterone to achieve healthy embryos and well prepared endometrium for embryo-uterine interaction culminating in pregnancy. Early pregnancy is that maintained by progesterone produced by the corpus luteum.[11] The endometrium during a normal pregnancy acquires a potential of the decidual

cell reaction[12] and the estrogen and progesterone are known to influence the above phenomena. The deciduogenic stimulus provided by the blastocyst results in hyper permeability of the endometrial capillaries to macromolecules also termed as blue reaction. Tubal transport of the ova in to the uterus, hyper permeability of the endometrial capillaries, are the parameters characteristics of early pregnancy.[13] Afternoon of day 4 is believed to be the time of the "estrogen surge", which is responsible for implantation on Day 5,[14] the surge occurs on Day 3,[15] but this was not a true surge. According to Ioannis Messinis,[16]the rate of ovarian secretion of estradiol rises from Day 2nd (1500-1700 hrs) to Day 3 and remains elevated for next few days. An injection of estrogen induces mitosis 24 hrs late in the uterus.[17] 100% post implantation loss, by this the drug indicates that the drug has a greater effect on differentiation and organogenesis and on the overall development of the embryo. *Ficus infectoria* was found to have no adverse effect on the periodically of the estrous cycle after given at a dose of 100 mg/kg body weight consecutively for two cycles in female albino rats. This appears to prove that the drug has completely reversibly of actions after the pregnancy. Anti estrogenic activity is usually determined by the ability of a compound to inhibit the increases in uterine weight induced by an estrogen. Moreover antiestrogens may be designated to those compounds which interfere with any of the actions of estrogen. The methods commonly used for the screening of possible antiovulatory substance vary considerably. In one test, the compound is fed to a group of mature female rats and the fertility rate observed. Some investigators follow the changes in the female rat and observed whether the estrous phase is suppressed by the test substance. These methods are not specific as ovulation cannot be predicted accurately in spontaneously ovulating mammal. Other testes have been devised where the gonadotrophin activity of the pituitary is determined in immature male and female rats. Ovulation is also detected by the presence of corpora lutea by serial histological sections of the ovaries. Absence of corpora lutea is an indication of antiovulatory activity. *Ficus infectoria* having Anti-implantation or abortifacient activities and estrogen is essential for implantation. This action of this drug may be due to inhibition of estrogen during the pre and post implantation period. The drug also seems to possess anti-ovulatory activity. All this may lead to disturbance in the hormonal balance between estrogen and progesterone leading to the contraception or may cause the abortion.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and the writing of the paper.

REFERENCES

1. Wright KP, Johnson JV. Evaluation of extended and continuous use oral contraceptives. Therapeutics and Clinical Risk Management, 2008; 10, 4(5): 905-11. PubMed PMID: PMC2621397.
2. Jain R, Muralidhar S. Contraceptive Methods: Needs, Options and Utilization. Journal of Obstetrics and Gynaecology of India, 2011; 02, 14, 61(6): 626-34. PubMed PMID: PMC3307935.
3. Jain S. Medicinal plants with potential anti-fertility activity: A review, 2015.
4. Pattar J, N B S, Vijaykumar M, Krishna S, Satyanarayana ML. Toxicological studies of FicusVirens in Wistar Albino Rats, 2012; 84-7.
5. Anandjiwala S, Bagul MS, Parabia M, Rajani M. Evaluation of Free Radical Scavenging Activity of an Ayurvedic Formulation, Panchvalkala. Indian Journal of Pharmaceutical Sciences, 2008; 70(1): 31-5. PubMed PMID: PMC2852057.
6. Kunwar R, Bussmann R. Ficus species in Nepal: A review, 2006; 85-97.
7. Agrawal SS, Alvin Jose M. Anti-implantation activity of H2 receptor blockers and meloxicam, a COX-inhibitor, in albino Wistar rats. The European Journal of Contraception & Reproductive HealthCare, 2009; 12, 01, 14(6): 444-50.
8. Shafiq N, Malhotra S, Pandhi P. Comparison of nonselective cyclo-oxygenase (COX) inhibitor and selective COX-2 inhibitors on preimplantation loss, postimplantation loss and duration of gestation: an experimental study. Contraception, 2004; 69(1): 71-5.
9. Agrawal SS, Jose MA. Anti-ovulatory activity of H2 receptor blockers in albino rabbits – A preliminary study. The European Journal of Contraception & Reproductive Health Care, 2011; 04, 01, 16(2): 142-6.
10. Kachroo M, Agrawal SS. Isolation, Characterization and Anti-Fertility Activity of the ActiveMoiety from the Seeds of Ensete superbum Cheesm (Banakadali), 2009; 01, 01: 9.
11. Kamboj VP, Dhawan BN. Research on plants for fertility regulation in India. Journal of Ethnopharmacology, 1982; 09, 01, 6(2): 191-226.
12. FINN CA. ENDOCRINE CONTROL OF ENDOMETRIAL SENSITIVITY DURING THE INDUCTION OF THE DECIDUAL CELL REACTION IN THE MOUSE. Journal of Endocrinology, 1966; 36(3): 239-48.
13. HUMPHREY K, MARTIN L. THE EFFECT OF OESTROGEN AND ANTI-OESTROGENS ON OVUM TRANSPORT IN MICE. Journal of Reproduction and Fertility, 1968; 15(2): 191-7.
14. KRAICER PF, MARCUS GJ, SHELESNYAK MC. STUDIES ON THE MECHANISM

OF DECIDUALIZATION. Journal of Reproduction and Fertility, 1963; 5(3): 417-21.

15. YOSHINAGA K. EFFECT OF LOCAL APPLICATION OF OVARIAN HORMONES ON THE DELAY IN IMPLANTATION IN LACTATING RATS. Journal of Reproduction and Fertility, 1961; 2(1): 35-41.
16. Messinis IE. Ovarian feedback, mechanism of action and possible clinical implications. Human Reproduction Update, 2006; 12(5): 557-71.
17. TACHI C, TACHI S, LINDNER HR. MODIFICATION BY PROGESTERONE OF OESTRADIOL- INDUCED CELL PROLIFERATION, RNA SYNTHESIS AND OESTRADIOL DISTRIBUTION IN THE RAT UTERUS. Journal of Reproduction and Fertility, 1972; 31(1): 59-76.