INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR)
ISSN: 2231-6078
6(1): April.-June.: (2016), Sppl. 1-8

Research Article

EVALUATION OF ANTIFERTILITY ACTIVITY OF PROGESTERONE EGG ALBUMIN MICROSPHERES IN WISTAR RATS

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Abstract

One of the biggest issues facing some countries, especially developing and less developed ones is the population expansion. It's still a problem even with the abundance of contraceptive options available. As a result, a contraceptive dosage that is easy to self-administer, affordable, effective, and safe is required. The goal of the current study is to assess the antifertility effects of progesterone egg albumin microspheres made by heat denaturation with glutaraldehyde acting as a cross-linker. The microspheres were injected via the nasal route, and their effects were compared to those of a typical intramuscular NE injection. Esterus phase, antiovulatory, antiimplantation, and antifertility activities were carried out in the work that was undertaken. Changes in the weights of the uterus and ovaries, as well as in the levels of glycogen and cholesterol, were also noted.
Ovarian and uterine histology was done to assist our research. The prolonged prostertus phase (from 2.05±0.52 days in the control group to 6.21±0.28 days in the drug-treated group) and reduced metasterus phase (compared to the control group) in the vaginal smear study results suggest that the granulosa cells' inability to produce estrogen, which is necessary for the maturation and differentiation of ovarian follicles, is the cause. Along with the graafian follicles, ruptured follicles, and corpus luteum mentioned above, ovarian sections in the control group also revealed these signs, indicating that ovulation had occurred and that there was no hormonal imbalance. During laparatomy, the drug-treated group demonstrated a considerable reduction in the number of implant sites, from 26 in the control group to 14 in the drug-treated group. However, the number of viable foetuses decreased from 7.89±0.27 in the control group to 3.58±0.38 in the drug-treated group. Compared to the control group, the weight of the live fetus in the drug-treated group decreased considerably, from 2.38 ± 0.25 gm to 1.46 ± 0.16 gm. The antifertility effect of progesterone egg albumin microspheres was demonstrated by the biochemical parameter measurements, which revealed a significant rise in cholesterol content in the uterus of the drug-treated group when compared to the control group. The histology research provided more evidence in favor of this conclusion.

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Introduction :

Contraception is the need of the day for controlling the population explosion in various developing countries. The choice of contraceptive therapy, especially the female –used till date is not to the people full contentment. This is because each of the available female-controlled contraceptive measures suffers some of the major drawback. Therefore there is a need for contraceptive dosage from that is readily self-administered, inexpensive, efficient and safe. Acceptability of contraceptive in terms of social, religious and cultural background is another factor of consideration. Currently, oral contraceptive and intra-uterine devices are most popular and used by millions of women. But no method of contraception is 100% effective.

In recent literature, the intranasal route of drug administration for systemic effects has been proven to be very effective.. A study was performed to evaluate the effects of intranasal administration of norethisterone on folliculogenesis, cervical mucus, vaginal cytology, endometrial morphology and reproductive-endocrine profile in women (Anand Kumar et.al, 1991).Levonorgestrel with mucoadhesive agents administered nasally for contraception in experimental rats was superior for maintaining effective drug concentrations over an extended period of time when compared with the presently available orally administered form (Shahiwala Aet al,2004).Albumin microcapsules and microspheres cross linked with glutarldehyde and 2, 3 butanedione were investigated for progesterone delivery by Orienti and Zecchi (1993), Jameela S. R) et al (1998) studied in laboratory animals that glutarldehyde cross-linked chitosan microsphers are long acting biodegradable carriers suitable for controlled delivery of many drug. An erythrocyte model has been used to investigate the membrane activity of various agents which have been employed as enhancers of intranasal drug absorption (G. Chandler et al, 1995).The present work focuses on developing in- vivo method to study of the antifertility activity of Progesterone egg albumin microspheres in rats.

Materials and Method

Preparation of Progesterone Loaded Egg Albumin Microspheres for controlled release

Albumin microspheres were prepared by a multiple emulsion and suspension crosslinking techniques.(Orienti V. etal.1993). Progesteron (40 mg) was dispersed in 1 ml of olive oil. This dispersion was mixed with 2 ml of 6% w/v egg albumin aqueous solution. The mixture was stirred at 250 rpm for 20 minutes to produce o/w emulsion. The emulsion was added to 5 ml of olive oil and the mixture was stirred again at 500rpm for 2 minutes to attain the corresponding o/w/o multiple emulsions.

1. The mixture was added drop wise using dropping funnel to olive oil with continuous stirring at 1000 rpm for 15 minutes.
2. The microspheres were stabilized by adding 0.1 ml of 25% w/v glutarldehyde solution heated at 37^0C with uninterrupted stirring for 60 minutes or by adding emulsion system to the preheated olive oil (100 ml) at 120º C drop wise with uninterrupted stirring for 30 minutes. Additional heat treatment was given to prepare microspheres
3. The preparation was cooled to 20˚C and centrifuged at 700 rpm. The supernatant was decanted. The obtained microspheres were washed with liquid paraffin and twice with ether to get a free flowing and discrete product. After that microspheres were suspended in anhydrous ether and stored at 5^0C in an airtight container.

by keeping them at 120ºC for 60, 90,120 minutes). The microspheres were characterized for particle size, swellabilty, drug-polymer compatibility and in-vitro release profile.

IN-VIVO STUDY

The in-vitro studies were found to be satisfactory and therefore based on the in vitro outcomes; in-vivo studies were then performed.

Procurement, selection and preparation of animals

Adult albino rats (Wistar strain, weighing 150-200 g) were used in this investigation. They were obtained for the study from Animal House- Deptt of Pharmacy, K.N.Polytechnic, College, Jabalpur. The animals were stabilized for 1 week. Animals were acclimatized to laboratory conditions before the experiment. They were housed under standard condition of temperature (24°C ±10º C), relative humidity (65±10%), light and dark cycle (14:10 h). They had free access to food and water and fed with standard pellet food. All the experiments were carried out between 09:00 and 15:00 h. All the animals were carefully observed for the development of any toxic signs or symptoms at different time intervals of 12 hrs, and then daily for period of 14 days. No abnormal sign of symptoms were observed. No mortality was observed. The initial body weight of each animal was recorded. Only female rats with normal estrus cycle were selected for the evaluation of antifertility activity.All experimental procedures were carried out in strict accordance with the guidelines prescribed by the CPCSEA and experimental protocols were approved by Institutional Animal Ethics Committee of Pharmacy Dept. K.N.Polytechnic, College, Jabalpur M.P.

Experimental protocol

18 female albino rats selected for present investigation were divided in three groups and treated as follows-The first group (n=6) served as control and received the vehicle only (saline p.o daily).

- The second group (n=6) served as standard. Each rat in this group was injected intramuscularly with a dose of 5 mg/kg body weight (NE injection-200mg/ml, Zydus,G. Remedies)one time during study.
- The third group (n=6) served as test group. Each rat in this group received equivalent dose of 2µg/kg body weight egg albumin microspheres form with 2 ml sterile saline suspension through nasal route once daily.

1.Exterus cycle

The phases of estrus cycle of each female rat used for the study of antiovulatory effects was determined by observing vaginal smear daily between 9 and 10 AM continuously for 15 days. Vaginal smear was prepared by introducing a drop of distilled water into the vagina with the help of a dropper, collecting back and placing it on a clean slide after adding a drop of glycerin (Shanbhag Tet al, 1990). The prepared smear was examined microscopically under low power for different types of cells. There are three types of cells such as cornified(keratinized) ,non-neucleatedcells, epithelial cells and leucocytes cells are found in the vaginal smear during a normal rat estrous cycle. The presence and absence of these cell types and the relative proportion of each cell type determine the stages of the estrous cycle. Presence of large number of nucleated epithelial cells indicated the proestrus phase (Fig-1a). Estrus phase was confirmed when the smear showed more than 50% cornified non-neucleated keratinized cells (Fig-1b).Metestrus phase was indicated by the presence of many neuclocytes and scattered smaller numbers of non-nucleated epithelial cells in the smear (Fig-1c).If the majority of cells are leucocytes, then it was labeled as diestrus phase(Fig-1d).

2. Antiovulatory activity

This was studied by observing the effect of progesterone microspheres on the duration of phases of estrus cycle, estimation of ovarian weight and cholesterol and histological study.The animals were fasted overnight.

An observation of different phases of esterus cycle of female rats was used for the study of antiovulatory effect. It was determined by observing vaginal smear from each rat every morning between 9-10 a. m. daily to cover three regular estrous cycles for 15 days. On the 16thday, the rats from each group were anesthetized and sacrificed by cervical dislocation(Jyoti Shresthaet al, 2010). The uteri dissected out were freed from extra deposition and their surrounding tissues removed. The uteri were blotted on filter paper and quickly weighed on a sensitive balance before fixing. The uterus and ovary were first fixed in formalin and then processed for histological studies. Paraffin embedded uteri were cut at 6 µm by using rotatory microtome. The sections were stained with haemotoxylin–eosin solution, examined and photographed for histological observations. Right ovary from each rat was processed for cholesterol estimation and another for histotological study.

Cholesterol estimation

Ovarian tissues was weighed and homogenized in cold saline to get uniform suspension. Cholesterol was estimated using manual kit supplied by Micro Science Limited, Jabalpur.

3 Antifertility and Antiimplantation activity

Female rats exhibiting three consecutive regular estrus cycles were chosen for the study. Female rats of proestrus phase were cohabited with male rats of proven fertility in the ratio of 2:1in the evening. The female rats were examined in the following morning between 9 and 10 a.m. for evidence of copulation. The animal which showed thick clumps of spermatozoa/ vaginal plug in vaginal smear were separated from the male partner and divided into 3groups (n=6). The day when spermatozoa detected in vaginal smear was considered as day 1 of pregnancy. The rats were weighed daily and observed any untoward effects. On the day 20 of gestation, each rat was laparotomised under light ether anesthesia. The uterine horns were exteriorized and incision at the greater curvature of the horns. The latter was examined for sites of implantation, resorption and number of live fetuses in both horns of the uterus. Th eweights of the fetuses were also determined. Uterine horns were frozen for biochemical estimation for cholesterol and glycogen level. The observations of the drug-treated groups were compared with control group.

Cholesterol estimation

Uterine tissues was weighed and homogenized in cold saline to get uniform suspension. Cholesterol was estimated using manual kit supplied by Micro Science Limited, Jabalpur.

Glycogen estimation

Uterine tissues was weighed and homogenized in cold saline to get uniform suspension. Glycogen was estimated using manual kit supplied by Micro Science Limited, Jabalpur.

Statistical analysis

The data ware statistically analyzed and expressed as mean ± SEM. The variance between control and experimental values was analysed by student's't' test. $p<0.05$ was considered significant.

RESULTS

Antiovulatory activity

The esterus cycle was found to be irregular and disturbed. Almost all the treated groups showed prolonged prostertus phase. Because of it duration of cycle was extended from 2.05±0.52 days in control to 6.21±0.28 days in drug treated groups. It was revealed that there was a significant decrease in the median duration of the esterus and metasterus phase of the drug treated group as compared to control group. However, there was no significant change in the duration of the disterus phase between control and treated group as shown in Table-I.

In drug treated groups ovarian weight decline significantly from 45.23 ± 1.05mg to36.25 ± 0.79 mg as compare to control group as shown in Table-II.

The mean ovarian cholesterol level in the drug treated group significant increases 0.42 ± 0.12mg to 0.57 ± 0.05 mg as compare to control group as shown in Table-II.

Section of the ovaries in the control group showed primordial, primary, graafian, ruptured follicles, and corpus luteum in the ovarian stroma along with blood vessels, adjacent fibrous area with mature adipose tissue. In drug treated group ovarian sections showed only primordial, primary, and secondary follicles but were devoid of graafian follicles,ruptured follicles, and corpus luteum(Fig-2).

Antifertility and Antiimplantation activity

The drug sample did not cause any abortion and vaginal bleeding. All the females in control group became pregnant with significantly increasing weights 207. 75 ± 3. 08 gm from to 186. 00 ± 2. 28 gm as compare to drug treated group as shown in Table-V.

The pregnant animal did not show any sign of toxicity. On laparatomy, drug treated group showed significant inhibition of number of implant site from 26 to 11 as compare to control group and also less no of live foetuses from 6.37±0.18 to 2.68±0.28 as compare to control group as shown in Table-III-IV

In drug treated group weight of live foetus decline significantly from 2. 66 ± 1.02 gm to 1. 65 ± 0. 35 gm as compare to control group as shown in Table-V

- In the present investigation, an increase in the cholesterol content from 15.89±0.4 mg to 19.25±0.3 mg but decline in the glycogen content from8.86±0.5 to7.92±0.3 of the uterus of the drug treated group was observed as compare to control group as shown in Table-VI

Table-I- Effect of control and drug samples on esterus cycle of female albino rats.

Reproductive cycles of female mice	Control (Group 1) (days)	Standard (Group 2) (days)	Treated (Group3) (days)	% Change in treated rats
Prosterus	2.05±0.52	5.35±0.28	6.21±0.28	+202.92
Esterus	3.18±0.47	2.05±0.35	1.02±0.07	- 67.92
Metasterus	4.15±0.19	2.18±0.15	2.28±0.09	-45.06
Diesterus	5.86±1.16	3.95±0.05	5.24±0.28	+ 10.58
Total period of cycle	15.24 ±1.02	13.53 ±0.17	14.75±0.40	-3.21

Values are expressed as means ± SEM for six rates in each group. P<0.05 vs control (student's't' test)

Table II. Effect of Standard and test drug sample on ovarian weight and cholesterol level.

Treatment	Ovarian weight in mg/100g body weight	Cholesterol level in ovary (mg/50mg)
Control (Group 1)	45.23 ± 1.05	0.42 ± 0.12
Standard drug sample (Group 2)	37.19 ± 0.65	0.53 ± 0.09
Treated drug sample (Group 3)	36.25 ± 0.79	0.57 ± 0.05

Values are expressed as means ± SEM for six rates in each group. P<0.05 vs control (student's't' test)

Table III- Effect of control and drug samples on Anti-implantation Activity

Groups	No. pregnant/ No. tested	No. o dead rats	No. of rats showing implantations	Total No of implant-ations	Anti-implantation activity (%)
Control *(Group 1)*	5/5	0	5	26	NIL
Standard drug sample *(Group 2)*	3/5	0	3	15	42.30%
Test drug sample *(Group 3)*	2/5	0	2	11	57.69%

Values are expressed as means ± SEM for six rates in each group, P<0.05 vs control (student's't' test)

Table IV- Effect of control and drug samples on Anti-fertility Activity

Groups	No. pregnant/ No. tested	No. of dead rats	No. of live foetuses (LFN)	No. of dead foetuses	Anti-fertility Activity (%)
Control *(Group 1)*	5/5	0	6.37±0.18	0	NIL
Standard drug sample *(Group 2)*	3/5	0	3.69±0.25	0	40%
Test drug sample *(Group 3)*	2/5	0	2.68±0.28	0	60%

Table V- Effect of control and drug sampleson maternal and foetal weights.

Parameters	Control	Standard drug	Treated drug sample
Maternal weights(g)			
Day 1	159. 42 ± 1.69	155. 30 ± 1.25	150. 40 ± 1. 25
Day 7	175. 33 ± 1.98	158. 33 ± 2.98	154. 50 ± 2.95
Day 14	186. 50 ± 2.68	169. 00 ± 2. 37	168. 33 ± 1.85
Day 19	207. 75 ± 3.08	191. 00 ± 3. 12	186. 00 ± 2. 28
Foetal weight (g)	2. 66 ± 1.02	1. 79 ± 0. 48	1. 65 ± 0. 35
Placental weight(g)	0. 52 ± 0.56	0. 296± 0. 25	0. 18 ± 0. 52

Values are expressed as means ± SEM for six rates in each group, P<0.05 vs control (student's't' test).

Table VI- Effect of control and drug sampleson the Biochemical Parameters of the uterus of female rates.

Groups	Glycogen Mean± S.E.M	Cholesterol Mean± S.E.M
Control *(Group 1)*	8.86±0.5	15.89±0.4
Standard drug sample *(Group2)*	7.57±0.2	19.86±0.3
Test drug sample *(Group 3)*	7.92±0.3	19.25±0.3

Values are expressed as means ± SEM for six rates in each group, P<0.05 vs control (student's't' test).

DISCUSSION

Rats were used to test the effects of progesterone microspheres on histology, ovarian weight, cholesterol levels, and the length of the various estrus cycle phases. It is commonly known that exact equilibrium of the progesterone and estrogen is necessary for implantation, and that any disruption in this equilibrium may result in infertility (Psychoyos, 1966).

Numerous physiological, biochemical, morphological, and histological changes take place in the ovaries during the estrus cycle. Endocrine (prolactin, luteinizing hormone, and follicle stimulating hormone) and ovarian hormones (progestin, estrogen, and androgen) control follicular growth and ovulation. Several ovarian cell types, including the corpus luteum and the granulosa cells of mature follicles, produce ovarian hormones (Circosta C et al, 2001).

This resulted from an imbalance in endogenous steroid, protein, and hormone levels, or from the granulosa cells' inability to make the estrogen needed for the ovarian follicles to mature and differentiate. Along with the graafian follicles, ruptured follicles, and corpus luteum mentioned above, ovarian sections in the control group also revealed these signs, indicating that ovulation had occurred and that there was no hormonal imbalance.

The histology investigations provided additional evidence in favor of this conclusion (Fig-2).Gonadotrophic and steroidal hormones cause the weight of the ovarian tissue to grow during the estrus cycle. When compared to the control group, the weight of the ovaries in the drug-treated groups significantly decreased, indicating a decrease in the activity of the stroma, the follicle, and this decrease could be brought on by a luteal phase defect, which is also known as insufficient progesterone secretion by the corpus luteum or placenta. Abortion, abortifacient, and antifertility effects have all been linked to luteal phase defects (Schorge JO et al,2008).

A precursor molecule for the production of steroid hormones like estrogens, progestins, and androgens is cholesterol. The current study's substantial increase in the cholesterol content of the drug-treated group's uterus suggests that cholesterol is not used for steroidogenesis, which is necessary for maintaining pregnancy, potentially interfering with conception (Eik, Ne et al., 1962)& (Shivalingappa H,et al., 2002).

The main energy source stored in uterine tissue is glycogen, and hormone secretion affects the amount of glycogen in the tissue. According to the current study, a decrease in the glycogen content of the experimental animals' uteri results in a decrease in the amount of stored energy needed for gestation (Walas O.1952), (Christei, G. A.,1966), and (Montgomery, R., 1957).

CONCLUSIONS

In summary, each parameter examined in the conducted pharmacological investigation suggests that progesterone egg albumin microspheres administered via nasal delivery had antiovulatory, antiimplantation, and antifertility effects.The study's results show great potential for the

development of a novel drug delivery system in the field.

Acknowledgment

I am appreciative to Famy Care, Pharmaceuticals, Navi Mumbai, for donating a gift sample of progesterone, a type of contraceptive medication, to me.

REFERENCES

Anand kumar et.al., T. C. and, Shah R.S,Institute for Research in Reproduction (ICMR), India, Bombay- July 1991

Christei, G. A.(1966): Implantation of the rat embryo: glycogen and alkaline phosphatise.J.Report.Fertile,12:279-294, .(Paper published in journal)

Circosta C, Sanogo R. Occhiuto procera on estrous cycle and on estrogenic functionality in rats. Farmaco 2001; 56:373-8.

Eik, Ne ., K. B. and Hall.P. F. (1962): Proc. Sol.Exp. Biol. Med., 111:280.l.Lal. G. (1976): Evaluation of Post-Coital antifertility substances and their effects on histochemistry. Ph. D. Thesis University of Rajasthan, Jaipur (Thesis)

Jyoti Shrestha[1], Tara Shanbhag[1], Smita Shenoy[1], Arul Amuthan[1], Krishnananda Prabhu[2], Stuti Sharma[2], Samik Banerjee[1], Sajala Kafle[1]Antiovulatory and abortifacient effects of *Areca catechu* (betel nut) in female rats-386.Indian J Pharmacol 2010; 42:5:306-311. (Paper published in journal)

M Jameela S. R.et al J. Controlled Release, (1998).52, p17 -24.

ontgomery. R. (1957): 1957 Determination of Glycogen.Arch. Biochem., Biophy., 67: 378

Orienti V. and Zecchi V. Progestrone loaded albumin microparticles .J. Contol Rel, 27 (1993), pg 233-235

Psychoyos(1966) Recent Research on Egg Implantation. CIBA Foundation StudyGroups. P23

Shahiwala A,&Misra A,Fertility and Sterility, 81,, 2004,.,893-898.

Schorge JO, Schaffer JI, Halrorson LM, et al. Williams Gynecology. China: The Mc Graw-Hill companies; 2008. p. 137-40

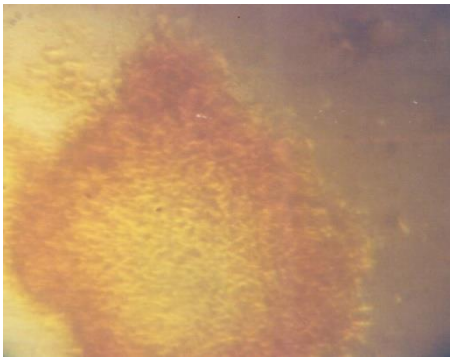
S. G. Chandler, N. W. Thomasand l. Illumctivity Nasal absorption in the rat: IV. Membrane activity of absorption enhancers,International Journal of Pharmaceutics,Volume 117, Issue 2, 18 April 1995, Pages 139-146

Shanbhag T, Bairy KL,Kulkarni DR, Drug screening and feasibility of intraperitonial route. Indian Drugs 1990,27:604-06

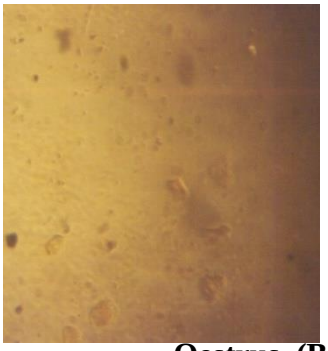
Shivalingappa H, Satyanarayan ND, Purohit MG, Sharanabasppa A, Patil SB. Effect of ethanol extract of Rivea hypocrateriformis on the estrous cycle of the rat. J Ethnopharmacol 2002;82:11.

Walas O. (1952): of Oestrogen on the glycogen content of the rat uterus.Acta Endoerm, 10:175-192.(Paper published in journal).

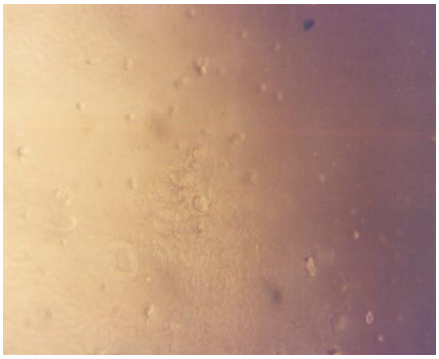
FIGURE-1 STAGES OF OESTRUS CYCLE.



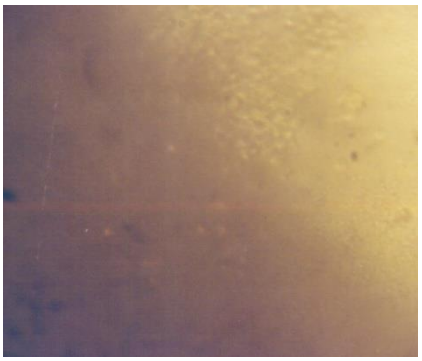
Pro-oestrus (A)



Oestrus (B)

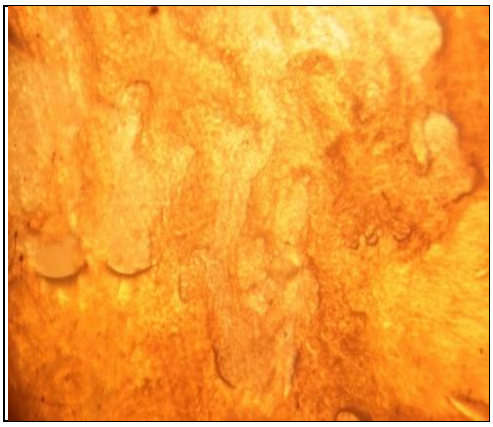


Metoestrus (late) (C)



Di-oestrus (D)

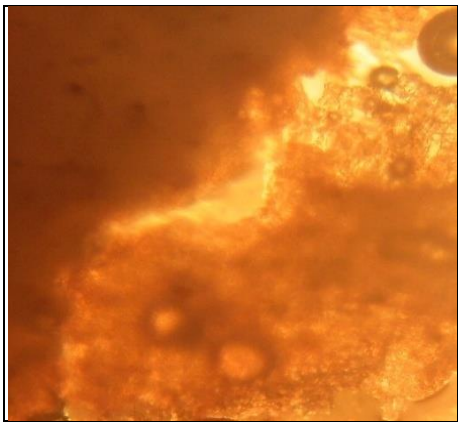
Fig 2.HISTOPATHOLOGICAL STUDY OF OVARY OF CONTROL GROUP AND TEST GROUP.



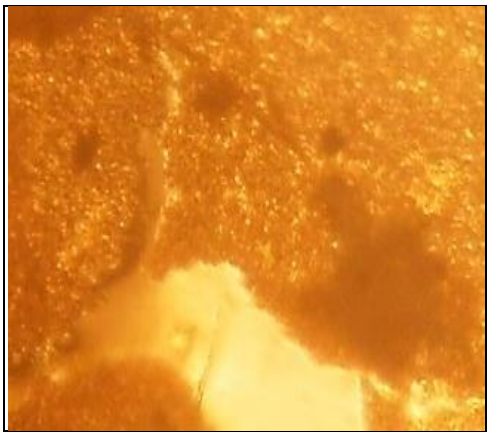
CONTROL

TEST

Fig 3. HISTOPATHOLOGICAL STUDY OF UTERUS OF CONTROL GROUP AND TEST GROUP.



CONTROL



TEST