MODE OF ACTION OF CENTCHROMAN AT VAGINAL AND OVARIAN LEVEL IN IMMATURE RATS

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Summary: Centchroman studied at various doses did not cause ovulation in immature rats as judged by morphological, histological and biochemical parameters whereas, 20 *mg/kg* dose caused early opening of vagina and cornification of the vaginal epithelial cells. However, no ovulation was detected by this regimen. No stimulatory effect was observed in the glycogen and cholesterol content in the ovaries of Centchroman treated rats. The effects on vagina persisted even in ovariectomised immature rats administered with Centchroman. The mode of various doses of Centchroman in immature rats as judged morphologically, histologically and biochemically has been discussed.

Key words: immature rat ovary centchroman gonadotrophin activity

INTRODUCTION

Recently, it has been reported by Roy *et al.* (6,7) that Centchroman induces ovulation in considerable number of amenorrhoic women by modulating hypophyseal - gonadotrophin action. Rama *et al.* (5) also confirmed gonadotrophin stimulating action of Centchroman in males by measuring elevated levels of serum LH and FSH in normal men. Action of gonadotrophins on the ovarian follicles has been worked out at cellular level (2, 10) and production of cyclic 3', 5' AMP in ovarian follicles after PMS gonadotrophin in immature rats ovary has been also reported (4,9). On the basis of above findings immature rats model was selected where maturation of the ovarian follicles and ovulation could be induced by Centchroman - a new property assigned by Roy *et al.* To confirm the above finding, present studies were taken up to induce vaginal opening and ovulations in immature rats by Centchroman.

MATERIALS AND METHODS

Immature rats (30 days old) of the Institute colony with closed vagina were divided in seven groups of ten rats each, group is as follows:

Group number	*No. of rats*	*Regime*
A	10	Normal physiological saline (control)
B	10	Pregnant Mare Serum (50 I.U. sacrified 72 hr later)
C	10	PMS+Follicle Stimulating Hormone (250 μg)
D	10	PMS+FSH+Centchroman (3 *mg/kg* body wt.)
E	10	Centchroman 3 *mg/kg* body wt. for 5 days
F	10	Centchroman 6 *mg/kg* body wt. for 5 days
G	10	Centchroman 20 *mg/kg* body wt. for 5 days.

The Centchroman was injected to the rats of groups E, F and G, whereas Pregnant Mare Serum (PMS) and Follicle Stimulating Hormone (FSH) was injected intramuscularly to the rats of groups B,C and D.

Vaginal opening of the treated groups of rats were observed closely and as soon as the vaginal opening was noticed smears were prepared immediately to study the nature of the cells coming in the vaginal secretions and were recorded. The animals were sacrificed later on. Uterine and ovarian weights were recorded carefully on a Roller Smith Balance. Glycogen (3) and Cholesterol (11) was estimated from the ovaries of the animals of each group. Tissues from the ovaries were fixed for histology and were stained with H & E. In yet another group of immature rats, ovariectomy was done and Centchroman in the dose of 20 *mg/kg* injected, to see its direct effect on vagina.

RESULTS

Table I shows that Centchroman in the doses of 3 and 6 *mg/kg* did not increase uterine and ovarian weights, 20 *mg* dose did not increase ovarian weight but uterine wieght increased significantly ($P<0.001$). Administration of PMS, PMS+FSH significantly increased uterine and ovarian weights ($P<0.001$). However. Centchroman (20 *mg/kg*) did not increase weights further to PMS+FSH group ($P>0.02$). Ovarian cholesterol decreased in all the groups whereas. glycogen registered no change except in group C (PMS+FSH) where it increased ($P<0.001$).

Table II shows early opening and mucification of vagina in PMS. PMS+FSH and PMS+FSH+ Centchroman group with the absence of cornified cells in the later group. Cornified cells were abundantly observed in PMS, PMS+FSH, and 20 *mg* centchroman group. Centchroman in the doses of 3 and 6 *mg* opened the vagina on 6th and 7th day of administration with epithelial cells and leucocytes in abundance in vaginal smears. No cornified cells were observed. In control rats, vaginae were opened on 11th and 12th day of the period of observation with mostly leucocytes in smear slides.

TABLE I : Effect of pregnant mare serum (PMS), follicle stimulating hormone (FSH) and centchroman on the uterus and ovaries of immature rats.

Sl. No.	Groups	Uterus	Ovary		
		Weight (mg) (mean±S.E.)	Weight (mg) (mean±S.E.)	Glycogen (µg/mg) (mean±S.E.)	Cholesterol (µg/mg) (mean±S.E.)
A.	Control (10)*	15.4±1.5	8.1±0.4	0.956±0.1	75.0 ±4.7
B.	Pregnant Mare Serum (PMS) 50 I.U. (10)	49.4±3.9	30.5±3.1	1.676±0.01	15.705±0.5
C.	Pregnant Mare Serum (PMS) 50 I.U. + Follicle Stimulating Hormone (FSH) 250 µg (10)	56.6±4.2	32.2±3.2	1.822±0.01	16.824±0.6
D.	PMS + FSH + Centchroman (3 *mg/kg*) (10)	34.9±2.2	28.3±3.0	1.265±0.01	13.216±1.7
E.	Centchroman 3 *mg/kg* (i.e. 0.1 *mg*/33 *gm*) (10)	18.9±1.2	7.2±0.8	0.952±0.1	9.963±0.57
F.	Centchroman 6 *mg/kg* (i.e. 0.2 *mg*/30 *gm*) (10)	18.3±1.0	8.9±0.6	1.038±0.01	22.942±1.7
G.	Centchroman 20 *mg/kg* (i.e. 0.6 *mg*/30 *gm*) (10)	23.5±0.4	7.1±0.4	1.117±0.09	18.870±0.8

*Number of rats.

Histologically, ovary of an immature rat shows presence of graffian follicles with several layers of granulosa cells, surrounded with thin elongated thecal cells. Vascular supply was not very well developed (Fig.1). In the ovariectomised rats, Centchroman (20 *mg*) induced vaginal opening on 6th day of treatment - thereafter the vaginal smear cytology showed mostly diestrus and proestrus, further substantiating a local centchroman effect in vagina (Table II).

In Centchroman treated ovaries of immature rats follicle size was much reduced but not the numbers, intrafollicular fluid was moreover absent and there were only one-two layers of granulosa cells, vascular supply was poor (Fig.2).

PMS and PMS+FSH treatment enhance the rate of proliferation organisation and maintenance of maturing follicles of the ovaries of immature female rats, at places antrum formation in PMS and ovulation in PMS+FSH groups was observed. Vascular supply was very well developed (Fig.3).

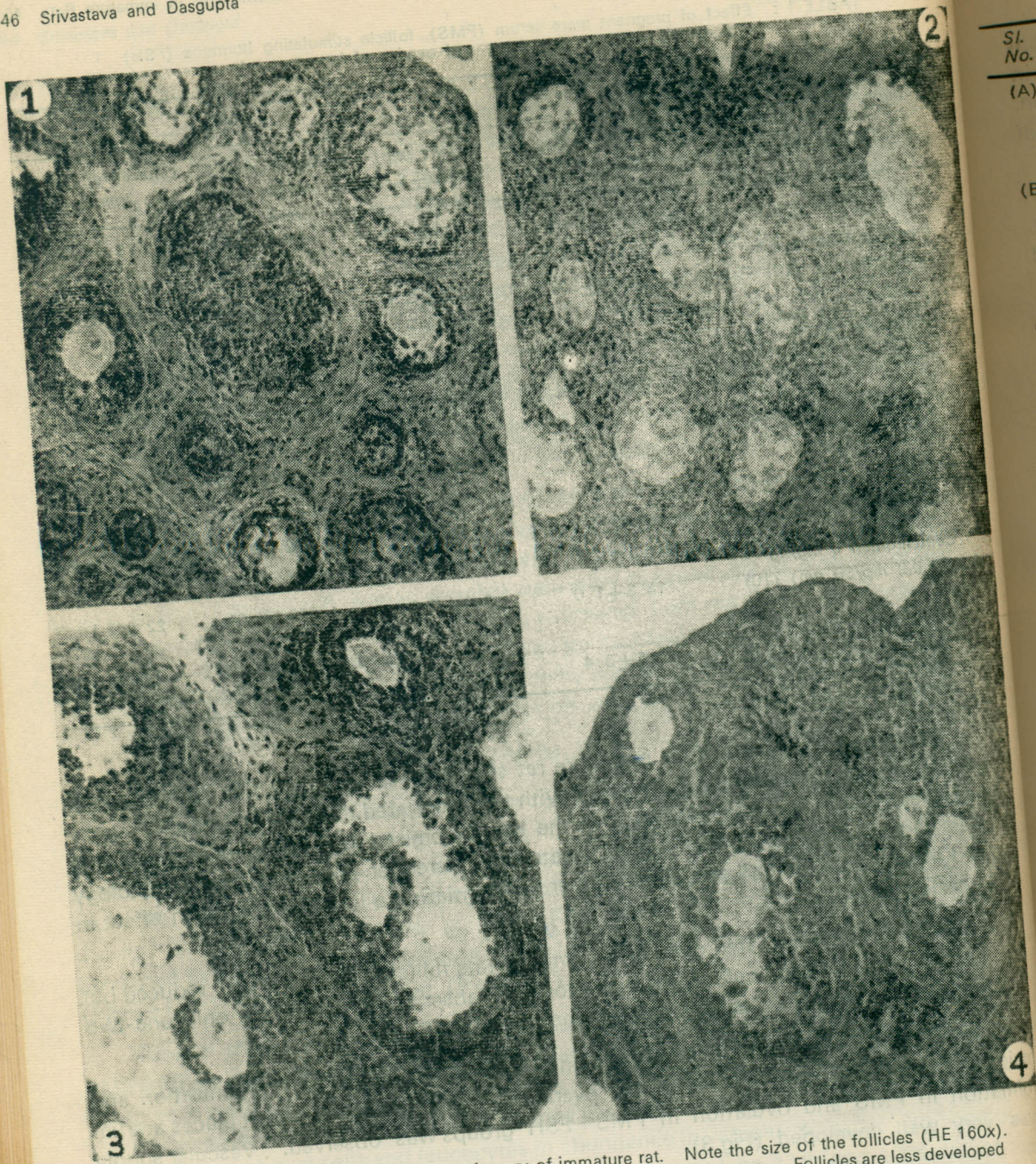

Fig.1 : Showing histological features of ovary of immature rat. Note the size of the follicles (HE 160x).
Fig.2 : Showing features of ovary of immature rat treated with Centchroman. Follicles are less developed
Fig.3 : Treated with PMS+FSH follicles are well developed, associated with antrum formation.
Fig.4 : Treated with PMS+FSH+Centchroman. Note developed follicles but no luteinization and antrum formation

TABLE II: Effect of pregnant mare serum, follicle stimulating hormone and centchroman on vaginal cytology and follicular maturation of ovaries in immature rats.

Sl. No.	*Groups*	*Vaginal cytology and day of vaginal opening*	*Histological observations*
(A)	Control	On 11th and 12th day vaginae were opened; with few leucocytes and epithelial cells, DIESTRUS STAGE. No cornified cells.	Size and number of follicles two-three layers of granulosa cell. Number varied (40-100) average intrafollicular fluid, basement membrane and vascular supply was maintained.
(B)	Pregnant Mare Serum Gonadotrophin 50 i.u.	Vaginae were opened on 5th day. Leucocytes, epithelial and few cornified cells were present, DIESTRUS, PROESTRUS.	Two-three times increase in size of the follicles, large amount of intra-follicular fluid, several layers of granulosa cells (150-1500), enrichment of antrum formation, maintenance of thecal layer, vascular supply well developed.
(C)	PMS + Follicle Stimulating Hormone (250 μg)	Vaginae were opened in the evening of 4th day in 3 rats. In 7 rats it opened on 5th day. Leucocytes, epithelial and cornified cells present. DIESTRUS, PROESTRUS, ESTRUS.	All the above observations were noticed as in B group. Ovulation also occurred in this group.
(D)	PMS+FSH+Centchroman (3 *mg/kg*)	Vaginae were opened on 5th day in 4 rats only with leucocytes and epithelial cells. In rest 6 rats vaginal mucification was seen on 6th day. DIESTRUS.	Follicles size less developed than FSH group, less amount of intra-follicular fluid, three-four layers of granulosa cells (150-300) few follicles with antrum formation, vascular supply well developed.
(E)	Centchroman (3 *mg/kg*, i.e. 0.1 *mg*/33 *gm*)	On 6th and 7th day vaginae were opened. Leucocytes and epithelial cells were present. DIESTRUS.	Size of follicle moderately enlarged, two-three layers of granulosa cell (30-60, 60-100) intra follicular fluid in few cells, vascular supply poor.
(F)	Centchroman (6 *mg/kg*, i.e. 0.2 *mg*/30 *gm*)	On 6th and 7th day vaginal cornification was observed, leucocytes and epithelial cells. DIESTRUS.	Picture was moreover same as in 3 *mg*/group.
(G)	Centchroman (20 *mg/kg, i.e.* 0.6 *mg*/30 *gm*)	On 6th and 7th day vaginal cornification was observed. Leucocytes, epithelial and cornified cells, were also seen. DIESTRUS, PROESTRUS, ESTRUS.	Size of the follicles much reduced but number of follicles did not change when compared to controls, intra-follicular fluid absent, one-two layer granulosa cells (40-80 cells) vascular supply poor.

DISCUSSION

Roy *et al.* (6) have observed modulating effect of Centchroman on hypophyseal gonadotrophin axis in rodents and recently Roy *et al.* (7) confirmed ovulation inducing property of Centchroman in anovulatory women. Typical effects of PMS as a gonadotrophin were observed in immature rat model in increasing weights of the ovary and uterus, premature opening of vagina, maturation and luteinization of the follicles, whereas, none of these gonadotrophin effects could be obseved in Centchroman groups. Due to early opening of the vagina of the immature rats it may be suggested that estrogen receptors for centchroman are lying abundantly in vagina (8); higher the dose of centchroman greater the response in vagina as evidenced by a good number of cornified cells in 20 *mg* centchroman group.

Histology of the ovary of centchroman treated groups revealed neither stimulation of

primary follicles nor luteinzing effect of the drug, but moreover ovary showed picture of an immature control rat (Fig 4.); details of which have been discussed in Table II.

Immature rats treated with PMS, PMS+FSH and centchroman showed a significant decrease in cholesterol content of ovaries suggesting a deesterification of cholesterol ester in the ovaries either by increased rate of hydrolysis or a decreased rate of esterification. Behrmans Armstrong (1) also found similar decrease in cholesterol content of ovaries of immature rats treated with LH.

Increase in glycogen content of the ovaries of PMS, PMS+FSH is a typical gonadotrophic effect, whereas no change in centchroman group suggest a lack of stimulatory effect.

So, on the basis of data obtained in this experiment in an immature rat model treated with different doses of Centchroman, it may be interpreted that it does not stimulate gonadotrophin secretion but its action may be stimulatory at vaginal or ovarian level (8) and Centchroman can not be considered as gonadotrophin stimulator. This is further supported by our observations in the ovariectomised group, where Centchroman treatment resulted in early vaginal opening and changes in vaginal cytology. It would be wrong to conceive stimulation of ovaries by Centchroman, without observing earliest changes in the ovarian tissue as reviewed by Lindner *et al.* (2) are stimulation of the tissue content and release of cyclic AMP which ultimately results in the organisation, maintenance, antrum formation and ovulation of the ovarian follicles. Evidences are also on record that ovarian steroidogenesis by FSH and LH is mediated by cyclic AMP (2,4,9). So, it does not seem logical to assign centchroman a gonadotrophin stimulator, in absence of all the changes which became prerequisite for the action of gonadotrophin in an immature rat modal experiment. However, the possibilities of centchroman working directly at ovarian level cannot be omitted.

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