

Effect of coumestrol on the histological feature of ovary of pups of F1 generation delivered from exposed mothers at different days of gestation

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ABSTRACT

Phytoestrogens are the plant substances, which are structurally and functionally similar to steroids and produce estrogenic effects. It is well established that phytoestrogens when administered orally caused remarkable effects on the reproductive organs in females. It is also known that female rats, which consume more phytoestrogen for longer period, suffer from sterility. In the present study, efforts have been made to assess the effect of coumestrol on reproductive organs when exposed *in utero* in mother and its effects on the development of postnatal pups. The administration of coumestrol during different days of pregnancy induced remarkable changes in the histological features of ovary of female rats of F₁ generation even at the age of 6 months.

Figure : 00

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KEY WORDS : Coumestrol, Graafian follicle, Phytoestrogens.

Introduction

Phytoestrogen exert estrogenic influence and affect morphology and histology of the ovary and uterus. These substances are known to induce uterotrophic effect, which lead to induce remarkable cellular changes in the ovary and uterus. Although the effect of phytoestrogens on cellular organization of ovary and uterus is well documented in intact and new born rats however, it is not yet known what happens at the histological status of the ovary and uterus of female rats which are delivered from mothers exposed to such substances especially by the coumestrol during pregnancy. Exposure of these substances may occur under two different conditions *i.e.* exposure before and after placenta formation. Such studies certainly may clarify whether the phytoestrogens are transported from mothers to foetus through placenta and if phytoestrogens are crossed through the placenta, what is the possibility and duration of retentions of such substances in the reproductive tract of delivered rats at different age. Therefore, in the present study efforts have been made to assess the effect of coumestrol on reproductive organs when exposed *in utero* in mother and its effects on the development of postnatal pups.

Material and Methods

To carry out the experiment, control and experimental groups were taken into consideration and were administered different doses at different intervals as per experimental protocol.

Result

Control

(A) 2 Months

The histology of ovary of control rat at 2 months showed normal features . All cellular components appeared normal. A secondary graafian follicle showed well developed oocytes surrounded by a single layer of cells of membrane granulosa along with central cavity . The cells of corpus luteum appeared normal.

(B) 6 months

Histological features of ovary of control rat at the age of 6 months showed normal features like developing graafian follicles, loose stroma with visible stromal septae. The recently form corpus luteum was also seen . The luteal cells showed anastomosis and most of them showed vacuolation. Arrangement of luteal cells was loose .

Experimental Group

(i) 5µg/kg (Group 1 and 2)

Histological features of the ovary of 2 months old rats delivered from mothers consumed 5µg/kg of coumestrol on day 10th and 13th of pregnancy showed normal features. The stroma showed the presence of stromal septae. The developing oocyte appeared normal and surrounding luteal cells appeared normal. Presence of stromal septae were also observed in 6 months old rats. When the period of consumption of coumestrol was made on day 15th and 18th of gestation, ovary of 2 months old pups showed some minor changes. At 6 months duration ovary did not show follicles and stromal cells. In cavity of follicle RBC were visible.

(ii) 25 µg/kg (Group 1 and 2)

When 25 µg/kg dose of coumestrol was administered in pregnancy on 10th and 13th day, delivered pups at 2 months of age showed remarkable features of ovary and increased vascularity. In 6 months old rat luteal cells showed vacuolation and compactness. Stromal septae were observed.

When the coumestrol was administered on 15th and 18th days of pregnancy the delivered pups at 2 months of age showed changes in the histological features of ovary. At 6 months of age, degeneration was severe. The ovarian histological structures were disorganized and there was undifferentiation in graffian follicles and the entire ovarian mass was filled with blood cells. In the cavity of follicles the R.B.C. were visible.

Discussion

Histology deals with the cellular organization of organs and if any disorder occurs, it undergoes alterations and pathological symptoms are induced. Under the administration of estrogenic substances initially biochemical constituents are altered but later on changes are also induced in the histological components and finally the patho-physiological changes are induced. Ovary and uterus are the major organs in the female reproductive systems, which are affected with the administration of steroidal compounds especially estrogen and progesterone. Even the phytoestrogens are well known to induce changes in these organs.

Enormous literature is available which illustrated that administration of synthetic estrogen to cyclic rats induced remarkable alteration in the histological feature on the uterus and less in the ovary¹. Characteristics features in the ovary include the enlargement of blood vessel, stromal septae and luteolysis of corpora lutea. Phytoestrogens and other plant extract of estrogenic activity have been reported to induce histological changes in ovary and uterus.

An investigator³ has reported that administrations of ethanolic extract of *Ferula jaeschkeana* at single or multiple doses have increased the number of corpora lutea, vascularity, formation of inter stromal septae. Degenerative changes in the follicles have also reported under chronic administration of extract. A worker⁵ has reported the effect of ethanolic extract of *Crotalaria juncea* on ovary and uterus of rats. Authors reported that administration of extract at 1/10th of LD₅₀ dose for 1, 2 and 4 weeks, caused degeneration of follicle, cells of membranagranulosa were deformed and luteal cells were disorganized and diffused. When the extract was administered at 1/50th of LD₅₀ dose for 1, 2 and 4 weeks degenerated changes in the ovary were observed. Under the dose (1/10th and 1/50th) duration (1, 2 and 4 weeks) uterus showed remarkable changes. The height of luminal epithelium was increased and stroma was loose. It was reported that when 50% ethanolic extract of *Nerium odorum* was administered for 10 days the ovarian histological features were not changed, however, when the period of administration was extended upto 30 days, the estrogenic influence of the extract was prolonged reported that *Ferula jaeschkeana* caused anti fertility activity in laboratory animals and its extracts in various organic solvents like ethanol, benzene and chloroform prevented implementation in rats⁶. A worker⁷ reported that early postnatal treatment with androgen estrogen showed anovulatory sterility associated with polyfollicular ovaries in female rats. Neonatally treated mice with coumestrol after the age of 13 months showed persistent vaginal cornification, cervicovaginal pegs and down growths, uterine squamous metaplasia haemorrhaging follicles of ovary².

Changes in the histological features of the ovary under the influence of coumestrol treatment at 10th and 13th day (Group-1) of gestation at 5 and 25 µg/kg doses were observed. When pregnant rats were treated with 5 µg/kg dose ovarian histology of rats of F₁ generation showed changes like deformed wrinkled oocyte, presence of stromal septae were also observed in the ovary of 6 months old rats. When pregnant rats were treated on 15th and 18th day of gestation with the 5µg/kg dose, the ovary of delivered pups showed similar changes as that of Group-1. Additionally the luteal cells were enlarged and vacuolation was increased and cells of membranagranulosa were loosely arranged. At later duration, RBC was visible in follicles. 25µg/kg dose of coumestrol also showed similar changes in rats of Group 1 and 2 however, the magnitude of changes is severe. Additionally at 6 months of age ovary showed severe disorganized features and entire ovarian mass was filled with blood cells.

The administration of coumestrol during different days of pregnancy induced considerable changes in the histological features of ovary of female rats of F₁ generation even at the age of 6 months, although the effect was more prominent at the age of 2 month. Additionally

increase in vascularity and, haemorrhagic follicles were also observed in the present study. It is also interesting to note that out of two different doses of coumestrol used in the present study, the smallest dose of 5µg/kg is sufficient to induce alterations in animals of F₁ generations.

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