



*ijcrr*

Vol 04 issue 05

Category: Research

Received on:17/01/12

Revised on:01/02/12

Accepted on:08/02/12

## STUDY OF OESTROUS CYCLE AND HISTOMORPHOMETRY OF OVARY IN DMPA AND A POLYHERBAL DRUG TREATED ADULT FEMALE ALBINO RATS

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### ABSTRACT

The main aim of the present work is to study the effect of DMPA and a polyherbal on the estrous cycle and to see the morphometrical changes in the ovary and the serum hormone levels of progesterone and estrogen in adult female albino rats. Animals were divided into four groups groups I as control. Group II,III and IV animals were treated with Injectable contraceptive DMPA at a dose of 0.3mg/kg.body wt./day/animal for one complete estrous cycle (four consecutive days at same interval everyday). Every day the vaginal smear was taken by cotton swab method and stained to see the cyclicity. On the fifth day, Group II animals were sacrificed. Group III animals were treated with polyherbal drug at a dose of 500mg/kg.body wt./day/animal for next one consecutive cycle and were sacrificed on 9<sup>th</sup> day. Group IV animals were withdrawn with the injectable contraceptive and from the 5<sup>th</sup> day to 9<sup>th</sup> day animals were maintained till day 9 and they were also sacrificed on 9<sup>th</sup> day. Animals of all the groups were sacrificed by euthenesia method and ovaries were collected after proper perfusion and fixed in 10% formalin for histological and histomorphometric study. Control group of animals showed regular estrous cycle with four stages – proestrous,estrous,metestrous and diestrous. DMPA treated group of animals showed prolonged diestrous stage. Polyherbal drug treated group showed a recovery to the normal cyclicity whereas the Group IV animals though there was a recovery it was very slow and in some animals the stage remained in diestrous stage itself. Ovary showed atretic follicles and more of corpus luteum when compared to control and group III animals showed more of mature and secondary follicles when compared to group IV animals.

**Keywords:** oestrous cycle – DMPA – polyherbal drug – histomorphometry

### INTRODUCTION

The menstrual cycle in women has been disturbed by various stress factors like chemical, physical, hormonal imbalance, emotional, psychological etc., All these disturbances lead to many changes in their reproductive cycle which includes irregular menstrual cycle, ovarian disturbances, etc.,

Various treatments have been taken by those women to regularize and normalize their disturbance in the reproductive cycle and reproductive organs and many of them end in vain. The main aim of the present work is to study the stress related changes occurring in reproductive cycle and the ovarian changes observed and a trial has been taken to observe the recovery of the animals by a poly-herbal drug formulation. The work has been carried out as trial in

animal study using adult female albino rats as the estrous cycle can be compared with the menstrual cycle in human.

( Short,1972)

#### MATERIALS AND METHODS

Adult female albino rats weighing about 110-120gms were selected for the present study. Animals were procured after the approval of CPCSEA (No.01/002/2000), (No.01/010/2003).

Demedroxyprogesteroneacetate (**DMPA**) which is commercially available in the name of Depo-provera was used as a chemical stress to induce disturbance in the estrous cycle in animals. A polyherbal drug formulation containing *Crocus sativus*, *Cinamonumcamphora*, *Abroma augusta*, *Vitisquandragularis*, *Saracaindica* was used to observe the recovery of the animal in its reproductive changes. These herbs were believed to have uterotonic effects (Thirugnanam,1994;

Shanmugam,1989;Warrier,1994,Sangeetha, Mathur, Prakash,1989;Murugesamudaliar,1998; Kirtikar&Babu,1999)

Animals were divided into four groups of six animals each. Animals were caged in such a way three female and one male in the same cage with a meshed partition. All the animals were monitored for their normal estrous cycle by doing vaginal smear for two consecutive cycles and confirmed with their normal cyclicity. Then the animals were grouped and experimented.

**Group – I** Control; **Group – II** DMPA treated; **Group III** – Polyherbal drug treated; **Group IV** – Withdrawal of DMPA. Group I animals were maintained with the normal feed and water till the end of the experiment. Group II animals were treated with DMPA at a dose of 0.03mg/kg.body wt./day/animal. This is to induce a disturbance in the estrous cycle of the group II animals (Amatayaku,1979). Group III animals were treated with

polyherbal drug formulation from the day 5 after they were treated with DMPA same as like group II animals. It was given at a dose of 500mg/kg.body wt./animal. Polyherbal drug was given for the next following estrous cycle. Group IV animals were treated with DMPA same like group II, drug was withdrawn from 5<sup>th</sup> day and the animals were monitored from day 5 till the end of the experimental period.

Tissues were subjected to histological study using routine Haematoxylin and Eosin stain and Histomorphometric study using Ocular and Stage Micrometer. Volume of the follicles, corpus luteum, were studied using reticule (Bloom&Fawcett,1968). Progesterone and Estradiol Hormonal assay were done using RIA kit (Coat-a-count method) of M/s Diagnostic Products Corporation (DPC), USA (Berquist et.al., 1983).

#### Methods

Control group of animals were subjected to vaginal smear study at a regular interval of 6 hours every day till the end of the experiment. Group II animals were treated with DMPA intramuscularly every day at a dose of 0.3mg/kg.body wt./day/animal for one complete estrous cycle at regular interval (Everett,1996). The first injection was given at 6pm once identified with its proestrous stage. On the fifth day the same time all the animals were sacrificed. Group III animals were treated with DMPA in the same way simultaneously along with Group II animals and from the fifth day for the next consecutive cycle they were treated with the polyherbal drug formulation orally at a dose of 500mg/kg.body wt./day/animal for four consecutive days. On the 9<sup>th</sup> day all the animals were sacrificed. Group IV animals were also treated with DMPA same like group II and after the 4<sup>th</sup> day, the animals were left free to monitor their recovery without any drug administration. On the 9<sup>th</sup> day group I, III and IV were sacrificed and

tissues were collected for the study of all parameters.

Animals were sacrificed by euthanasia. First animals were anaesthetized and were then sacrificed using overdose of chloroform. Then the animals were perfused properly using normal and formal saline. Ovaries were removed and fixed in 10% formalin for morphology, histology and histomorphometric studies. Ovaries were stained by routine Haematoxylin & Eosin method (Bancroft, 1982). Histomorphometric studies were done on follicular and the luteal size (Hirshfield & Midgley, 1978a).

### **Observations**

Observations were made in all the four groups of animals on the following aspects Vaginal cytology, Morphology, Histology, Morphometry & Histomorphometry and progesterone and estradiol assay.

**Vaginal cytology:** In the control group, the vaginal cytology exhibited all the regular four stages of estrous cycle—proestrous, estrous, metestrous and diestrous at regular intervals as per the literature study (Long & Evans, 1922). All the stages existed as per the indicated duration. Vaginal smear showed different types of cells at different stages (Mandl, 1951) Proestrous stage contains nucleated cells of different sizes and shape. Estrous stage showed cornification and squamatization of cells as a result of which the nucleus were seemed hidden and were not identified. Also the cells existed either single or in cluster of two or three cells. The vaginal liquid appeared clear and watery. In metestrous stage the cells got infiltrated with leucocytes due to which the periphery of the cornified cells were bounded by leucocytes. The diestrous stage had only leucocytes and none other cells were identified. This stage is referred to as resting stage where no reproductive activity will take place. The vaginal fluid appears

highly viscous and milkfish white in appearance. The duration of each stage had been shown in the tabulated column.

In the DMPA treated group, the vaginal smear showed only a prolonged stage of diestrous stage as a result which it contains only leucocytes and none other cells. That indicates the animal remains in the resting stage (Bhowmik & Mukherjea, 1988). In Polyherbal drug treated group, vaginal smear showed the recovery of different stages of estrous cycle that were identified with the control group. Cells of different types like nucleated in proestrous stage, squamatized cells in estrous stage were identified. In the group IV, after the withdrawal of DMPA the vaginal smear showed recovery of the normal cycle but the duration seemed to be longer than the polyherbal drug treated group. All the vaginal observations were shown in the tabulated column and compared by the bar diagram.

**Morphology :** Group I animals showed small oval ovary where as in group II the ovary became enlarged and irregular shaped structure. Group III and Group IV animals showed a varied size and shape when compare to group II animals.

**Histology :** In group I animals, the ovary showed follicles of different stages – primary, secondary, mature and graffian follicles (Mandl, & Zukerman, 1952). Corpus luteum found to be present more in number in diestrous stage as it a resting stage. In group II animals, almost all the ovary showed more number of corpus luteum than the follicles and the size of the corpus luteum also found to be bigger than the group I animals (Oateberg, 1979). In group III and IV animals, there was a considered increase in the number of follicles when compared to group II animals. There was difference in the follicular existence in group III and group IV animals. Histological studies were shown in the photographs.

**Histomorphometry:** Studies like size of the follicles of different stages, size of the corpus luteum, and volume of the ovarian tissue were tabulated and represented by bar diagrams. Follicular & Luteal diameters were measured and compared by bar chart.

**Hormonal study :** In the control group of animals, the progesterone estrogen levels fluctuate during different stages of estrous cycle (Nequin, Alvarez & Schwartz, 1979). In DMPA treated group, the animals remained in prolonged diestrous stage and the hormonal assay were done at the diestrous stage in all the group of animals (Page & Butcher, 1982). The hormonal changes were tabulated and represented by graphical diagrams. Group III & IV animals show a recovery to their normal cyclicity and that of the group III is faster and regular in recovery than the group IV animals which are slow in recovery and also some animals remained in diestrous stage itself.

### CONCLUSION

Group I animals showed normal estrous cycle with the four stages as per the literature study. Ovaries showed different stages of the growing follicles and the size of the corpus luteum remained as same as per the literature study. Group II animals showed a prolonged diestrous stage where the vaginal cytology smear showed the existence of leucocytes only. Ovary showed extensive number of corpus luteum. Ovarian follicles remained atretic condition and few primary follicles were identified. Group III animals which were treated with polyherbal drug formulation showed a gradual and fast recovery to normalcy with the evidence of the presence of cornified epithelial cells, nucleated cells and leucocytes at specified stages. Ovaries showed the gradual existence of follicles of different stages and the corpus luteum. In group IV, vaginal smear showed the

recovery from the prolonged existence of diestrous stage, but in a slow manner and in some animals the recovery were not observed in the experimental period. Ovaries started showing few secondary follicles and one or two mature follicles. From the above study, it was clearly noticed that there was drastic effect of the usage of contraceptive (DMPA) on the estrous cycle and eventually disturbed the normal histology and histomorphometry of ovary. The treatment of the polyherbal drug on the contraceptive treated animals seemed to be very effective in regularizing the estrous cycle and subsequently produced changes in bringing back the normal histology of the ovary and it was proved by the histomorphometric studies. The withdrawal of DMPA also showed some effect in bringing back the normal cycle of the animal but not as effective as group III and also found to be less when compared to normal group. The serum progesterone and estrogen levels also found to be regularized in group III which were remaining in a single concentration in group II. They found to be more or less same in both Group III & IV.

### ACKNOWLEDGEMENT

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors/editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

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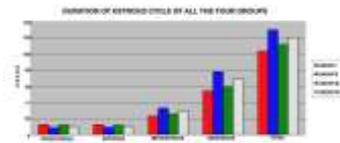
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Caging of Animals Stages of estrous cycle in all the four groups Control Group



PROESTROUS ESTROUS METESTROUS DIESTROUS GROUP II GROUP III  
GROUP IV

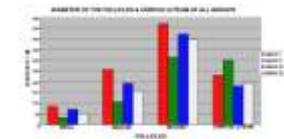
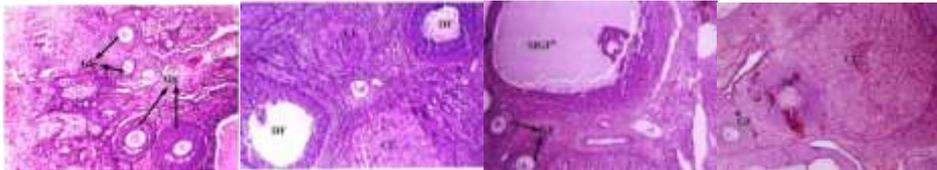


Duration of different stages of estrous cycle of all the groups were compared by Bar diagrams.

Histology of Ovary in all the four groups

GROUP I GROUP II GROUP III GROUP IV

Histomorphometry-comparison



Progesterone and estrogen in all the four groups

