Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3(3): 49-52

Received: 14-04-2015
Accepted: 17-05-2015

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Effect of Progesterone on Morphometry of the Stromal Cells of Goat (*Capra hircus*) Ovary

Sharma RK, Saini S and Ashraf H

Abstract
The present study was aimed to demonstrate the changes in morphometry that occur in the stromal cells of goat ovary due the supplementation of different concentrations of exogenous hormone i.e. progesterone, *in vitro.* For this purpose tissue fragments devoid of follicles and corpus luteum were dissected and treated with no hormone (control), 50 ng/ml and 100ng/ml of progesterone for 4 hours and 8 hours each.

Keywords: Morphometery, Stromal, Ovary, Progesterone

1. Introduction
A key modulator of normal reproductive functions, the steroid hormone, progesterone is associated with sexual responsiveness which includes ovulation, uterine and mammary gland development and the neurobehavioral expressions [1, 2]. In mammals, ovary is the major site for synthesis and secretion of progesterone giving rise to cyclical fluctuations in the levels of this hormone in the circulation [3]. Thus, in the complex regulation of normal female reproductive function, progesterone may be considered as a key component.
Ovarian stromal cells are active and influence the function of follicles. Essential for the organization of the follicle basement membrane, ovarian stromal cells in rat ovary at the neonatal stage, have their role in the formation of primordial follicles [4]. Albeit at different levels, granulosa cells, thecal/stromal cells also secrete progesterone [5, 6]. Nuclear staining for progesterone receptors was observed in the surface epithelium, cortical tubules, rete ovarii, follicle cells, thecal cells, luteal cells, granulosa cell cords and ovarian stroma [7, 8]. Early follicular growth may be regulated by progesterone via stromal and thecal cells [9]. In humans, the possibility of a paracrine action of ovarian steroids on follicles and corpora lutea, mediated through stromal steroid receptors and steroid-linked local factors, has been suggested [8]. Unlike the well characterized progesterone responsive tissue, it has been difficult to determine whether progesterone influences ovarian function because the ovary synthesizes progesterone in high amounts. However, Rothchild [10] put forth the concept that progesterone has an intraovarian site of action. Various research works have been done on the morphological changes occurring in different intraovarian tissues due to the action of different steroids. To our knowledge no work has been done to analyze the histomorphological changes occurring in goat ovarian stromal cells due to action of progesterone. Thus, the aim of the present study was to establish the effect of different concentrations of progesterone on the histomorphological changes in goat ovarian stromal cells.

2. Materials and methods
Caprine ovarian tissue culture:
Ovaries from adult cycling goats *(Capra hircus)* were collected and brought to the laboratory at 0 ^{0}C in normal saline from the slaughter houses of the adjoining areas of Kurukshetra (29^0 6'N, 76^0 5'E) in the month of February to April. The ovaries which had larger areas of stromal tissue were selected and washed with 70% alcohol for sterilization. After removing the outer surface epithelium under the view of zoom stereo microscope small fragments (<2-4mm thick) of stromal tissue excluding the follicles was microdissected from the ovaries and immediately placed in culture. The caprine ovarian fragments were cultured in 4 well plates (Nunc, Rosklide, Denmark) in 0.4 ml Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics (penicillin 100 IU/ml, streptomycin 100 µg/ml,

gentamicin 25 U/ml) at 37 ^{0}C in a humidified incubator under an atmosphere of 5% CO_2 and 95% air. Four to five ovarian fragments were made to culture in each well. The cultures were treated with no hormone (control group), progesterone 50ng/ml and 100ng/ml for 4 hours and 8 hours each. Each experiment was replicated at least 3 times and the data obtained was analyzed using the Graph Pad Prism (version 5.01). Experimental results were presented as mean ± SEM(standard error mean).

3. Histology

After the specified time period the cultured ovarian fragments were collected and placed in Bouin's fixative to examine the morphology of stromal cells after treatment. Post fixation the tissues were processed for paraffin embedding. The paraffin blocks were sectioned at 5 µm thickness with rotary microtome. The obtained sections were then stained with hematoxylin and eosin and photographed at 1000X with stereozoom microscope

Measurements were standardized using the stage micrometer at the same magnification. In each ovarian cross-section, the length and width of the stromal cells were measured. Morphometrical measurements were obtained from multiple cross-sections, of the goat ovary.

4. Results

In the present study an attempt has been made to study the changes in histoarchitecture and dimensions of goat ovarian stromal cells due to different concentrations of progesterone exposure for different time durations. Many morphometric changes were induced in the stroma of normal cycling goat ovary when treated with 50ng/ml of progesterone for 4 hours. An increase in length as well as width of these cells was observed. With supplementation of progesterone (50ng/ml) for the exposure duration of 4 hours, the length of the stromal cells increased from 5.65±0.2835 to 6.35±0.2542 and width from 1.35±0.1094 to 1.95±0.1141(Fig. 1b, Table 1 & 2), both of which were not statistically significant. In case of progesterone, 50ng/ml for 8 hours duration, the recorded variations in the dimensions of stromal cells were statistically significant ($P<0.05$) ie. The length increased from 7.2±0.2128 to 7.85±0.1313* and width from 2.05±0.1535 to 2.55±0.1141* (Fig. 2e, Table 1 & 2).

As the dose level was increased from 50ng/ml to 100ng/ml a marked increase in the dimensions of stromal cells was revealed. During 100 ng/ml progesterone treatment for 4 hours the length of stromal cells increased from 5.65 ± 0.2835 to 12.15 ± 0.5725** and width increased from 1.35 ± 0.1094 to 3.0 ± 0.156** (Fig. 1c, Table 1 & 2), which were statically significant. Length of stromal cells in case of 100 ng/ml progesterone for 8 hours, increased from 7.2±0.2128 to 12.6±0.6505** and width from 2.05±0.1535 to 2.95±0.1698** (Fig. 2f, Table 1 & 2). Variations recorded during exposure of 100ng/ml progesterone for 8 hours' time durations were found to be statistically significant

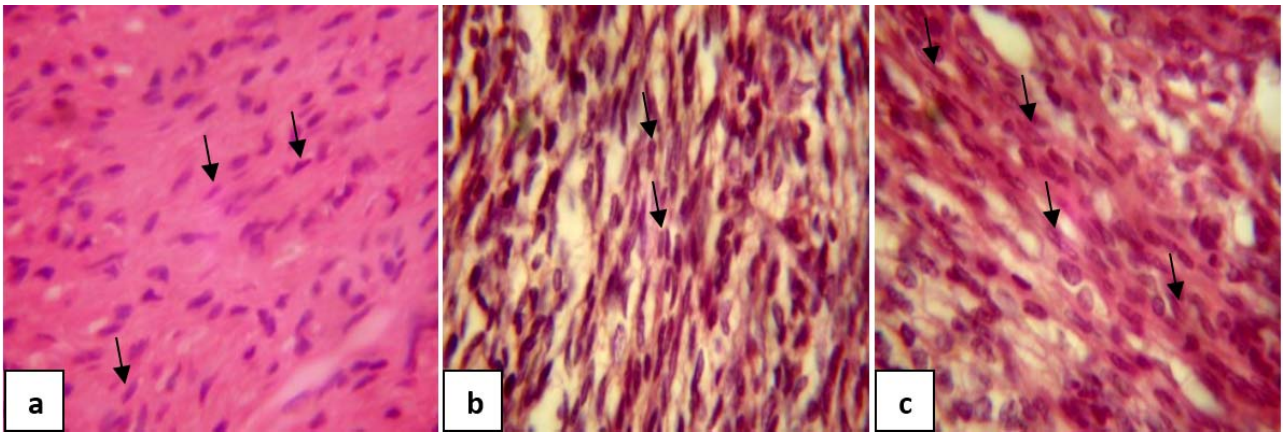

Fig 1: Portion of goat ovary showing stromal cells a) control group with no hormone treatment showing small sized fibroblast like stromal cells. b) Stromal tissue treated with 50ng/ml progesterone for 4 hours, the stromal cells showed a small but non-significant increase in their size. C) Stromal tissue treated with 100ng/ml progesterone for 4 hours, the cells showed a significant increase in their morphometery (arrows).

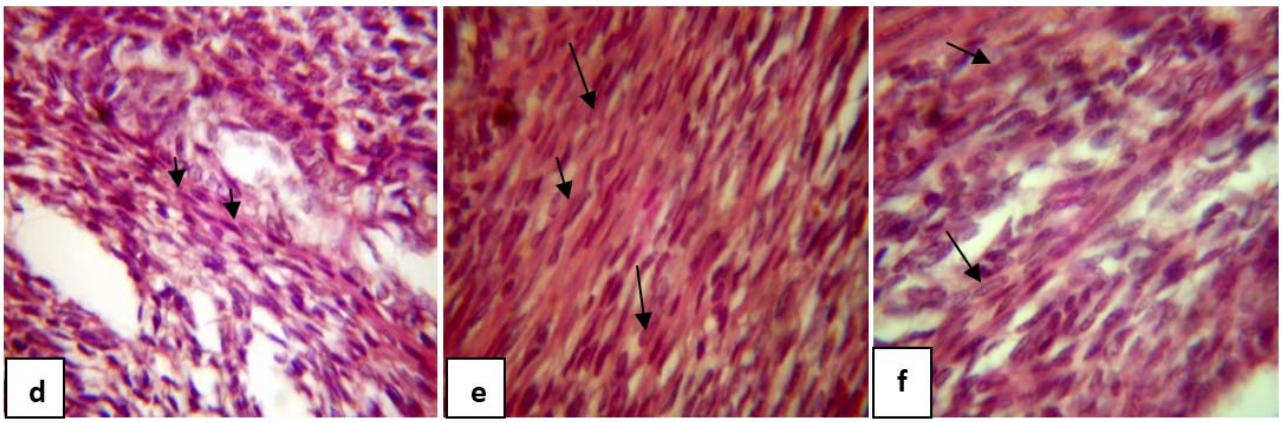

Fig 2: Portion of goat ovary showing stromal cells d) control group with no hormone treatment showing small sized fibroblast like stromal cells. e) Stromal tissue treated with 50ng/ml progesterone for 8 hours, the stromal cells showed a small but non-significant increase in their size. f) Stromal tissue treated with 100ng/ml progesterone for 8 hours, the cells showed a significant increase in their morphometery (arrows).

Table 1: Histomorphometeric data of effect of Estrogen and Progesterone on length (µm) of stromal cells during exposure of different doses at different time durations.

S.No.	Concentration and Time	Length (**µm**) of Stromal Cells
1.	Control, 4 hours	**5.65 ± 0.2835**
2.	50ng/ml, 4hours	**6.35 ± 0.2542**
3.	100ng/ml, 4hours	**12.15 ± 0.5725****
4.	Control, 8 hours	**7.2 ± 0.2128**
5.	50ng/ml, 8hours	**12.15 ± 0.5725****
6	100n/ml, 8hours	**12.35 ± 0.3574****

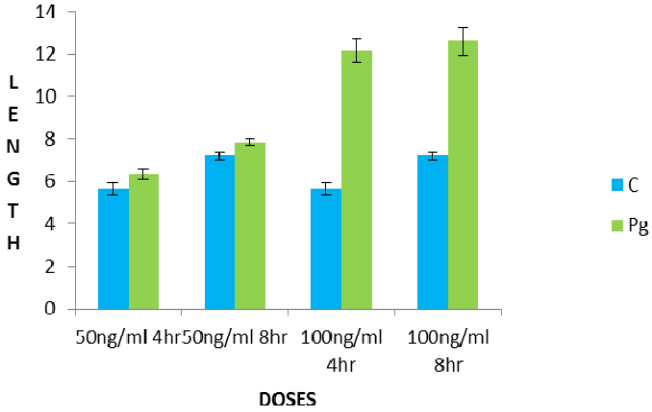

Fig 3: Effect of Progesterone on length of stromal cells at different concentrations and different time periods.

Table 2: Histomorphometeric data of effect of Estrogen and Progesterone on width (µm) of stromal cells during exposure of different doses at different time durations.

S.No.	Concentration and Time	Length (**µm**) of Stromal Cells
1.	Control, 4 hours	**1.35 ± 0.1094**
2.	50ng/ml, 4hours	**1.95 ± 0.1141**
3.	100ng/ml, 4hours	**3.0 ± 0.156****
4.	Control, 8 hours	**2.05 ± 0.1535**
5.	50ng/ml, 8hours	**2.55 ± 0.1141***
6	100n/ml, 8hours	**2.95±0.1698****

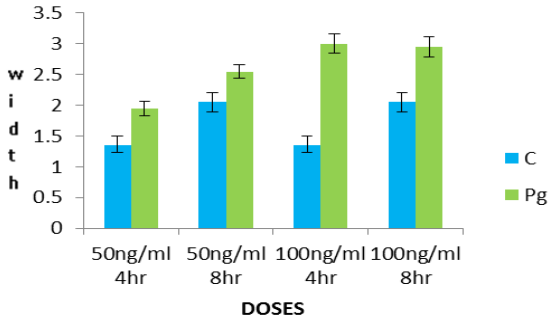

Fig 4. Effect of Progesterone on width of stromal cells at different concentrations levels and different time periods.

5. Discussion

A number of steroids are secreted by the ovary which includes pregnenolone, progesterone, 17_-hydroxyprogesterone, 17_-hydroxypregnenolone, dehydroepiandrosterone, androstenedione, testosterone, estrone, and 17_-estradiol [11]. Second-messenger signaling such as cyclic AMP (cAMP), cytokines, transcription factors and ovarian steroid production changes throughout the ovulatory cycle, qualitatively and quantitatively, under the control of various hormones, along which steroidogenic activity is also increased [12]. To the best of our knowledge, for the first time we report the histomorphometric changes occurring in goat ovarian stromal cells *in vitro*, due to the supplementation of different concentrations of exogenous hormones for different time periods. In the present study, stromal cells showed significant ($p<0.05$) hypertrophy with increase in dosage of progesterone and duration of time. These results are in accordance with the previous study in pinealectomized female rats which showed proliferation of stromal cells due to melatonin treatment [13]. The present study was further validated by the studies performed by Soares *et al.* [14] who established an increase in proliferation of ovarian stromal cells in female rats after giving them melatonin treatment. Various histological studies have demonstrated the presence of RNA and lipid bodies distributed in the compactly organized stroma showing their steroidogenic nature [15, 16]. Together these studies support the present study demonstrating increase in the morphometry of the stromal cells after progesterone treatment which is considered as one of the steroidogenic characters of the cells.

6. CONCLUSIONS

Overall, the histomorphometry revealed the fact that the exogenous in vitro supplementation of progesterone to the goat ovarian stromal cells in different concentrations leads to an increase in their size or hypertrophy which is related to increased steroidogenesis. Our study is helpful in specifically asserting the role of stromal cells in diverse ovarian functions. Therefore, the hypertrophy observed in this study suggests that these cells can be useful for *in vitro* culture of hormonal production.

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