# CONCENTRATIONS OF PROGESTERONE AND ESTRADIOL-17B IN THE FOLLICULAR FLUID OF OVARIAN FOLLICLES OF GOAT

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**Abstract**

*The aim of the present study was to quantify the Progesterone ($P_4$) and Estradiol-17β ($E_2$) in follicular fluid (FF) of medium (Gp-I; 3-5mm diameter) and large (Gp-II; more than 5mm diameter) goat ovarian follicles, by enzyme-linked immune sorbent assay (ELISA). Pooled samples (n=7) of FF from two groups of follicles were used for the study. The $E_2$ concentrations (ng/mL) in FF of Gp-I and Gp-II follices were 12.08±2.78 and 22.08±1.97 respectively. The $P_4$ concentrations (ng/mL) in FF of Gp-I and Gp-II follicles were 10.56±2.3 and 20.53±3.82 respectively. The $E_2$ and $P_4$ concentrations were significantly ($p \leq 0.05$) higher in the Gp-II follicles than the Gp-I follicles. Similarly, increase in $P_4$ concentration in Gp-II follicles might be due to the production of $P_4$ in the synthetic pathway of follicular production of $E_2$.*

**Keywords:** *Progesterone, Estradiol-17β and Follicular Fluid*

The steroid hormone concentration differs with stage of the estrus cycle, follicular size and degree of atresia (Kruip and Dieleman, 1985). In the late follicular phase estradiol production was increased in maturing follicle (Mc Lachlan *et al.,* 1987). Berisha *et al.* (2000) reported that comparatively healthy follicles had constant progesterone levels in the FF. The dynamics of antral follicles involve four phases namely recruitment, selection, dominance and atresia. Recruitment involved the follicular development where a cohort of small antral follicles was recruited by the ovarian pool and produce small amounts of $E_2$. After recruitment, a group of growing follicles that have not undergone atresia are selected and the selected follicles produce moderate amounts

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of $E_2$. Among selected follicles few follicles undergone atresia and few become dominant follicles. The dominant follicles that ovulate produce significant amount of $E_2$ (Senger, 2012). However, much study were not undertaken to analyze the FF of $E_2$ and $P_4$ in different sized goat ovarian follicles. So considering the importance of $E_2$ and $P_4$ in ovarian follicular dynamics this study was conducted to know the level $E_2$ and $P_4$ in different sized goat ovarian follicles.

## Materials and Methods

### *Sample collection*

Ovaries were collected from the healthy adult female Malabari goats immediately after slaughter from the local slaughter house. After removing the ovary from reproductive tract it was washed with the chilled normal saline (NS). Ovaries were transported to the laboratory within 60 min after slaughter in ice box with ice packs containing chilled NS.

The follicles were classified into two groups according to their diameter that is medium (Gp-I; 3-5mm diameter) and large (Gp-II; >5mm diameter). The follicular fluid was aseptically aspirated from the morphologically visible healthy follicles (well vascularized and having transparent follicular wall and fluid) of the same size by using the sterile 23 gauge needle fitted with the 2mL syringe. Follicular fluid from the same sized healthy follicles of same ovary was pooled together. The oocyte and the granulosa cells (GCs) suspended in the follicular fluid were removed by centrifugation at 12,000×g for 20 min. The supernatant follicular fluid was transferred to another sterile eppendorf tube stored at -20°C until determination of $P_4$ and $E_2$.

### *Hormonal assay*

Concentrations of progesterone and estradiol-17β in the follicular fluid of two different groups of goat ovarian follicles were determined by ELISA.

### *Hormonal assay-Progesterone ($P_4$)*

The follicular fluid progesterone concentration in the samples was analyzed using the commercial ELISA kit (Pathozyme® Progesterone, Omega Diagnostics Ltd.) according to the manufacturer's instructions with slight modification. The sensitivity of the Progesterone ELISA kit was 0.0625 ng/mL.

A standard curve was prepared by plotting the mean of the absorbance for each standard against its concentration in ng/mL for determination of progesterone value in the sample.

### *Hormonal assay-Estradiol-17β ($E_2$)*

Follicular fluid $E_2$ assay was validated by dilution test. 1:50 dilution was selected and all the samples were diluted and then used for the $E_2$ assay. The $E_2$ concentration in the samples was analyzed using the commercial ELISA kits (Pathozyme® Oestradiol, Omega Diagnostics Ltd.) according to the manufacturer's instructions with slight modification. The sensitivity of the Oestradiol ELISA kit was 1pg/mL. A standard curve was prepared by plotting the mean of the absorbance for each standard against its concentration (pg/mL) for determination of estradiol-17β value in the sample.

### *Statistical analysis*

Statistical significance between the two groups was calculated by independent t-test using the software Statistical Product and Service Solutions (SPSS), version 21.

## Results and Discussion

The average follicular fluid estradiol-17β concentration of Gp-I and Gp-II follicles

along with their standard error (SE) is depicted in Table 1.

The follicular fluid estradiol-17β concentration was found to be significantly ($p \leq 0.05$) higher in Gp-II than the Gp-I follicles.

McLachlan *et al.,* (1987) reported an increased Estradiol-17β concentration in the late follicular phase. Secretion of high concentration of estradiol is crucial for the successful follicular development and ovulation. The high FF estradiol level is the hallmark of dominant and preovulatory follicles (Kruip and Dieleman, 1985). In the present study it was observed that the follicular fluid estradiol-17β was significantly ($p<0.05$) higher in the Gp-II follicles than the Gp-I follicles. The result of this study was almost in agreement with Henderson *et al.* (1982) in bovine, Kalmath and Ravindra, (2007) in buffalo and Tungal *et al.* (2014) in goat follicular fluid. Similarly, Kruip and Dieleman, (1985) also reported that the steroid hormone concentration differs with stage of the oestrous cycle, follicular size and degree of atresia. Ireland *et al.* (1979) reported that large follicle contain highest estrogen concentration. Increase in concentration of $E_2$ with the follicle size might be due to increase in number of GCs with increase in the size of follicle as GCs are the principal site of aromatization activity in the pathway of formation of $E_2$. The mean of follicular fluid progesterone concentration of Gp-I and Gp-II follicles along with their standard error (SE) is depicted in Table 1. The analysis of data revealed that mean follicular fluid progesterone level was significantly ($p \leq 0.05$) higher in Gp-II follicles than the Gp-I follicles. Progesterone concentration is important for successful fertilization and the values are higher in normal fertilization compared to failed fertilization (Carpeintero *et al.* 2014). Further progesterone is the precursor of androgens, and it is an essential element for the production of estrogen. The follicular environment rich in all the three hormones that is estradiol, progesterone and testosterone is crucial for the development of good oocyte. A high rate of progesterone is crucial for determining good quality oocyte and for normal fertilization. They also observed higher pregnancy rate for the oocytes dipped in a follicular environment rich in progesterone, testosterone and estradiol. In future, estimation of FF hormone composition could be considered as an additional tool in oocyte selection. In the present study progesterone level in the follicular fluid was significantly ($p<0.05$) increased with size of the follicle. Our result was in agreement with that of Henderson *et al.* (1982) in bovine. However, Berisha *et al.* (2000) reported that healthy bovine follicles of all sizes had relatively constant progesterone level and Ireland *et al.* (1979) reported that small follicles contained the highest progestin concentration. In the present study we found higher concentration of both progesterone and estradiol-17β in Gp-II follicles than the Gp-I follicles. It might be due to the increased steroidogenic activity of follicular cells with increase in size of follicle. Similarly, increase in $P_4$ concentration in Gp- II follicles might be due to the production of $P_4$ in the synthetic pathway of follicular production of $E_2$.

The present study revealed that in case of Malabari goats progesterone and estradiol-17β concentrations in follicular fluid of healthy large

**Table 1:** Follicular fluid progesterone and estradiol-17β level in Gp-I and Gp-II follicles

| Hormone | Mean(±SE) follicular fluid hormone level (ng/mL) | | p-Value |
|---|---|---|---|
| | Group-1 | Group-2 | |
| Progesterone | 10.56 ± 2.3 | 20.53 ± 3.82* | 0.04 |
| Estrogen | 12.08 ± 2.78 | 22.08 ± 1.97* | 0.011 |

* - significant at 0.05 level

follicle (more than 5mm) were significantly ($p \leq 0.05$) higher than the medium (3-5mm) sized follicle.

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