Hormonal and Biochemical Profiles In Follicular Fluid of Unovulated Follicles in Superovulated Goats Ovaries

S. B. Deshpande*[1] and M. M. Pathak[2]

Reproductive Biology Research Unit
Anand Agricultural University, Anand-388110 (Gujarat)
* Corresponding author email: deshsat@gmail.com

Abstract

The composition of follicular fluid (FF) varies with the cyclical hormonal changes and developmental stage of follicle. During superovulation programme, the large number of unovulated follicles is a major constraint and affects adversely the embryo recovery. An attempt was made to study the hormonal and biochemical profiles of unovulated follicles in goat superovulated with either Synthetic FSH (133 mg Folltropin) or 1000 IU PMSG (Folligon). The study revealed that Progesterone and testosterone concentrations were significantly ($P<0.05$) higher in FF of unovulated follicles of suparovulated goats as compared to control. The total and free cholesterol levels were significantly ($P<0.05$) lower in FF of treated animals then control one. The Alkaline Phosphosphatase activity was recorded lower in treated group while Acid Phosphosphatase activity was observed significantly ($P<0.05$) high in FF of treated animals compared to control. Iron and zinc concentration were also recorded significantly ($P<0.05$) higher in FF of superovulated animal compared to control. The total protein concentration was observed high in FF of treated animals than control but the differences were statistically non significant. The concentration of estradiol 17 ß, Lactate dehydrogenase and Copper did not show much variation between treatment and control group.

Key words: Goat, Superovulation, Unovulated Follicles, Follicular Fluid Composition

Introduction

In the complete process of follicular development starting from primordial follicles to its ovulation or atresia a series of morphological, Physiological and biochemical events take place where various ovarian hormones play key role. In the synthesis of these hormones and their physiological actions need interaction and support of many enzymes, micro minerals and biochemical available in follicular fluid. The composition of follicular fluid varies with the cyclical hormonal changes and developmental stage of follicle. The theca cells convert cholesterol in to testosterone under LH influence, which later gets converted in to Estradiole 17 - ß in granulosa cells under the effect of FSH (Hafez, 1987). The exogenous gonadotrophins used for super ovulation facilitate to reverse the process of follicular atresia and increase the number of follicles destined to ovulate. However, inspite of exogenous LH injections, some of the follicles remain unovulated during normal and superovulation process (Baru, 1997 and Senthilkumar et al., 1998). The large number of unovulated follicles during superovulation is a major constraint in superovulation programme and affects adversely embryo recovery. Very few reports are available in hormonal and biochemical profiles of follicular fluid of normal cycling goat. However, no reports are available on hormonal and biochemical profile of follicular fluid of unovulated follicle in superovulated goats. Therefore, the present investigation was planned to study the hormonal and biochemical profiles in follicular fluid of unovulated follicles in superovulated goats ovaries.

Materials and Methods

Twenty four indigenous goats were super-ovulated using either 133 mg synthetic FSH (Folltropin-V) in 8 equal divided doses or 1000 IU PMSG (Folligon) as single injection. All the animals under treatment group received 7.5 mg prostaglandin F2 a (1.0 ml Prosolvin) either with 6th injection of Folltropin-V or 48 hrs after Folligon injection. All the animals received 750 hCG (chorulon) on the day of superovulatory estrus immediately after mating to ensure maximum ovulation. The superovulated goats were laparotomized on day 3 after mating. The follicular fluid was aspirated from unovulated follicles having more than 5 mm diameter present on the superovulated

1. Dept. of Animal Science, N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat
2. Associate Director of Research, Anand Agricultural University, Anand

ovaries and after pooling preserved at -20ºC till further analysis. The normal cycling goats were treated as control and were laprotomized on the day 3 of normal estrus and the follicular fluid was aspirated from the unovulated follicles. The follicular fluid was subjected for estimation of ovarian steroid hormone viz. progesterone (Coat A Count), testosterone (Coat A Count) and estradiol- 17 ß (Double antibody technique) by Radio Immunoassay using standard techniques. The biochemical's viz. total protein, cholesterol (total, free & ester), enzymes viz. Alkaline Phosphatase (AKP), Acid Phosphatase (ACP) and Lactate dehydrogenase (LDH) and micro minerals viz. Iron (Fe), Copper (Cu) and Zinc (Zn) were done using standard protocols. The data were analyzed as per Snedecor & Cochran (1980).

Results and Discussion

Hormones: The progesterone in the follicular fluid is synthesized as intermediary substances and plays important role in follicular development. In the present investigation, the concentration of progesterone in follicular fluid of unovulated follicules was observed significantly (P< 0.05) higher in superovulated group as compared to control group animals (Table- 1). The progesterone concentration in follicular fluid of control animals is in close agreement to findings of Sureshkumar (1990). The preovulatory LH surge inhibits aromatase actively of granulosa cells which inhibits estradiol synthesis. This alteration of ovarian steroidogenesis is due to inhibition of 17a hydrolase enzyme. So during preovulatory period LH surge may also stimulate more progesterone in follicular fluid (Guraya, 1986).

The Estradiole-17 ß levels in follicular fluid of unovulated follicles of superovulated goat were lower than control group but the differences were statistically non significant (Table-1). The values recorded for control animals are in close agreement with Suresh kumar (1990). The FSH and LH provide positive direct action in the synthesis of Estradiole-17 ß by involving theca and granulosa cells together (Short, 1962).

The testosterone concentration in the follicular fluid of unovulated follicles of superovulated goats was significantly higher than control animals in the present investigation (Table-1). The testosterone is a predominant androgen present in the follicular fluid and its concentration decreased with the follicular development by converting it into estradiole 17 ß towards final maturity (Wise, 1987). The higher level of testosterone and low level of estrodiole 17 ß in the follicular fluid of unovulated follicles in present investigation indicates towards failure of mechanism of conversion of testosterone into estradiole 17 ß and might have resulted into unovulated follicles.

Cholesterol: The cholesterol is present mainly as ester cholesterol (70%) and remaining 30% in free form. Apart from being the precursor for steroid synthesis, it is also important in formation of plasma membrane and hence might be involved in the growth and multiplication of granulosa and theca cells and also maturation of oocyte (Guraya, 1986). In the present investigation, the total and ester cholesterol levels were significantly (<0.05) higher in follicular fluid of unovulated follicles of control animals than the superovulated group. The finding of present investigation for control animals are in close agreement with Sureshkumar (1990). The ester cholesterol remains in the stored form and as per physiological condition and functional aspects it gets converted in to free form for steroidogenesis. The result of present investigation indicate that due to superovulatory treatment the mobilization in ester cholesterol to free cholesterol might have taken place and resulted in to high testosterone concentration.

Total Protein: The mean total protein concentration was significantly (P<0.05) higher in follicular fluid of superovulated goats as compared to control animals. The values recorded for control animals are in close agreement with Sureshkumar (1990) but are less than those reported by Siddhu et al. (1985). The follicular fluid provides osmotic pressure, steroid biding proteins and enzymes necessary for development of oocyte along with follicular development (Guraya, 1986). The higher concentration of total protein in follicular fluid of unovulated follicles in superovulated goats in present investigation might be to satisfy the high requirement of steroid binding proteins.

Enzymes: In the present investigation the activity of AKP was observed low in follicular fluid of superovulated animals as compared to control animal. However, the differences were statistically non significant. On the other hand, the activity of ACP was recorded significantly (P<0.05) higher in follicular fluid of treated animals then the control animals. The present findings AKP and ACP of control animals are in close agreement with Sureshkumar (1990). The AKP activity is associated with follicular atrophy (McNatty, 1978). The growing follicles had less AKP activity than atretic follicles. The high concentration of ACP in small antral follicles might limit their ability to respond gonadotrophin stimulation and leads to atresia (Handerson and Cupps, 1981). The lactate dehydrogenase (LDH) activity was recorded almost similar in follicular fluid of superovulated and control animals. Increased activity of LDH in follicular fluid may indicate early follicular degeneration (Wise, 1987). Almost equal LDH level in treatment and control group indicated that degenerative process was still not initiated in unovulated follicles even after 72 hours of superovulatory estrus.

Micro minerals: Micro minerals influence the

Table-1.Hormonal and Biochemical Profiles of Follicular Fluid of Unovulated follicles in Superovulated Goats

S.N	Parameter	Treatment		Control
		Folligon	Folltropin-V	
1.	Progesterone (ng/ml)	363.33a ± 33.62	301.25a± 17.64	276.66a± 19.93
2.	Estradiol 17-ß (ng/ml)	157.66a ± 10.52	168.88a± 8.40	174.75a± 10.02
3.	Testosterone (ng/ml)	194.44b ± 8.14	198.88b± 16.78	181.37a±7.16
4.	Total Protein (gm %)	14.22a ± 1.19	14.72a± 1.21	12.87a±1.12
5.	Total Cholesterol (mg %)	594.66a ± 27.84	602.88a± 13.64	672.37a±24.95
6.	Free Cholesterol (mg %)	86.00a ± 2.63	85.33a± 2.68	84.62a± 2.17
7.	Ester Cholesterol (mg %)	508.11a ± 26.76	517.55a± 13.20	587.75b±25.03
8.	AKP (KAU %)	18.77a ± 0.68	18.88a± 1.62	20.12a±0.95
9.	ACP (KAU %)	2.84b ± 0.20	3.10b± 0.16	2.31a±0.15
10.	LDH (IU/ml)	2.50a ± 0.10	2.33a± 0.18	2.24a±0.20
11.	Copper (µg/ ml)	0.95a ± 0.04	0.95a± 0.05	0.93a±0.08
12.	Iron (µg/ ml)	18.5b ± 1.80	15.8a± 1.20	13.1a±3.00
13.	Zinc (µg/ ml)	118.8b ± 9.60	124.4b ± 10.4	87.5a±9.90

physiology of reproduction through their action as metallo-proteins / metallo-enzymes. In the present investigation the copper concentration did not show any significant variation between superovulated and control animals. The copper levels observed in present investigation for control animals are in close agreement with Sharma and Vatsa (1998). The iron concentration in follicular fluid of unovulated follicles of superovulated goats was higher than control animals. The high level of iron in follicular fluid unovulated follicles of superovulated goats may be due to increased haemodynamic pulses in the vascular shunt of the developing follicles under influence of increased estrogen and biosynthetic activity of follicles (Sangha et al., 1993). The present investigation revealed that the mean zinc concentration in follicular fluid of unovulated follicles was observed significantly ($P<0.05$) higher in superovulated goats as compared to control animals.

Acknowledgement

Authors are thankful to authorities of Gujarat Agricultural University to provide necessary facility to conduct this investigation.

References

1. Baru, P. (1997): Biological and endocinological studies during embryo transfer in goats. Ph. D. Thesis submitted to Gujarat Agricultural University, S. K. Nagar, Gujarat, India.
2. Guraya, S. S. (1986): Biology of Ovarian Follicle. Springer Verlog, Berlin Heldelbera, New york
3. Hafez, E. S. E. (1987): Reproduction in Farm Animals. 5th edition. K. M. Varghese Company Post Box 7119, Bombay, India
4. Handerson, K. A. and Cupps, P. T. (1981): Acid Phosphatase Activity in Bovine Follicular fluid. *J. Anim. Sci.* (Suppl. 1): 329-332
5. McNatty, K. P. (1978): Follicula Fluid. In Vertebrate Ovary : Comparative Biology and Ovulation. R. E. Jones (Edi.) pp.215-259. Plenum Press. New York.
6. Sangha, G. K., et.al.(1993): Distribution of trace elements in blood and ovary during the estrous cycle and pregnancy in house rat. *Indian J. Anim. Sci.* 63(2): 142-145
7. Senthilkumar, et.al. (1998): Effect of porcine FSH and PMSG on ovarian response and embryo recovery in Tellichery goats. *Indian Vet. J.* 75(5):477-479
8. Sharma, R. K. and Vatsa, R. (1998). Biochemical Changes in Trace elements in Antral Follicles of Goats. *Indian J. Anim. Sci.* 68(4):330-331.
9. Short, R. V. (1962). Steroids in the Follicular Fluid of cow. *J Endocrino.* 23:401.
10. Siddhu, K. S., et.al.(1985). Electrophoretic characterization of Follicular Fluid proteins from goats (Capra hircus) ovary. *Indian J. Anim. Reprod.* 6(2): 41-48.
11. Snedecor, G. W. and Cochran, W. G. (1980): Statistical methods. Oxford and IBH Publishing Company, Calcutta, India.
12. Sureshkumar, P .K. (1990): Ovarian activity and hormonal aspects of Marwari goats in relation to estrous cycle phases. Ph.D. Thesis submitted to Gujarat Agricultural University, S. K. Nagar.
13. Wise, T. (1987): Biochemical analysis of Bovine Follicular Fluid. *J. Anim. Sci*. 64: 1153-1169.

* * * * * * * *