Ovarian Response and Profile of Plasma Sex Steroids in Goats Against Combined Administration of FSH and LH Isolated from the Pituitaries of Buffaloes

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ABSTRACT : This study was designed to record the ovarian response towards a combined administration of heterologous buffalo FSH (buFSH) and LH (buLH) in goats. The impact of such a treatment on ovarian structures and on the plasma profile of the ovarian sex steroids (estradiol 17-β and progesterone) was studied. The buFSH and buLH were isolated from the buffalo pituitaries involving a procedure of ethanolic extraction, acetone precipitation followed by metaphosphoric acid - ammonium sulphate fractionation. Both gonadotrophin samples prepared were found biologically active and potent. There was an increase in the total number of follicles in the treated group (12.66 ± 1.24) vis-a-vis the control group (8.50 ± 2.06). However, the percentage (51.48 ± 6.37) of large follicles were found reduced (23.74 ± 5.93) following the treatment. Again the number of corpora lutea were observed significantly higher (2.33 ± 0.47 C. L.) in the treated group than (1 C. L.) in the control group. The peak plasma estradiol-17β levels achieved, were much higher (17.16 ± 9.52 pg/ml) in the treated group, than the peak (7.22 ± 1.67 pg/ml) achieved in the control group. Similar trend was observed with respect to the progesterone levels (higher in the treated group). This study thus indicated that, a combined administration of heterologous buffalo FSH and LH to goats speeded up development of larger follicles nearing the ovulation stage. This population of the follicles subsequently got reduced and lead to the formation of the increased number of the corpora lutea observed in this study.

(Key Words: FSH, LH, Pituitaries, Buffalo, Goat, Follicles, Steroids)

INTRODUCTION

Gonadotrophins, FSH and LH promote formation of a larger number (than those usually formed) of ova. Usually, subcutaneous or intramuscular injections of PMSG or FSH are given to stimulate additional follicular growth. This is often followed by exopenous administration of LH or gonadotrophins with LH like activity to synchronize ovulation. Ovulation is generally induced in cows, sows and ewes through the release of the endogenous LH. But in mares, possibly in goats and in prepubertal animals, a supplementary treatment with LH or HCG may be needed (McDonald, 1980). Therefore, isolated gonadotrophins find great use for the reasons decribed above.

Present study was therefore, designed to pretest and study the ovarian response towards the administration of indigenously prepared FSH and LH from buffalo pituitaries in a smaller ruminant species (goat) before their evaluation in a more expensive, homologous species buffaloes.

MATERIALS AND METHODS

Isolation and preparation of buFSH and buLH

Buffalo pituitaries of either sex were collected immediately after slaughter from the local slaughter house and they were stored at −20℃ ± 5℃ till processed. Isolation of these gonadotrophins involved the following steps.

Preparation of ethanolic extract

Pituitary pulp was thoroughly mixed with cold acetate buffer (pH 4.5, 0.2M), with 40% ethanol. After shaking this material was centriguged and recentrifuged. Supernatant was washed with chilled acetone and precipitate was allowed to be formed at −15℃. Precipitate was dialysed against distilled water and then centrifuged. Supernatant was lyophilized.

Metaphosphoric acid and ammonium sulphate fractionation

Above extract, having both FSH and LH was dissolved in sodium phosphate buffer (pH 7.3, 0.025M) to

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get a protein concentration of 20 mg/ml, pH of this solution was adjusted to 4.2 with 0.035M metaphosphoric acid. It was centrifuged at 16,300 g and pH of the removed supernatnat was readjusted to 7.3 with in NaOH. Supernatant was dialysed against 0.5 saturated $(NH_4)_2SO_4$ buffered at 7.3 with 0.025M sodium phosphate. Cold centrifugation was given at 16,300 g. Supernatant containing the FSH fraction and precipitate containing the LH fraction were separated out.

The FSH fraction was dialysed against the dilute soluton of ammonium acetate and lyophilized. The LH fraction was dissolved in sodium phosphate buffer and was finally lyophilized to get the buLH preparation.

Bioassay of the prepared gonadotrophins

The finally prepared buFSH was assayed on the basis of the augmentation with HCG, following the method of steelman and Pohley (1953), FSH-P obtained from Schering Corporation, USA, Kenilworth, NJ07033 Animal Health Division was used as a reference standard for evaluating the biopotency.

Isolated buffalo luteinizing hormone (buLH) was assayed by the method of Loraine (1950), using immature white Swiss albino male rats.

Experimental animals

From the Divisional animal herd, eight normal cyclic, healthy black-bengal goats of 3-5 years were selected and kept under identical and standard conditions of hygiene, feeding and management.

Animals in the control group received only the solvent, in the same volume and using the same made of administration as for the treated group animals. Treated group animals received buFSH (doses equivalent to 20 mg of FSH-P) in four descending doses, for four days, starting from the 13th day of the estrus. A shot of PG (125 ug of carboprost) was given in the evening of the third day of treatment. Goats received buLH (doses equivalent to 500 I. U. of HCG) on the day of $PGF_{2\alpha}$ induced estrus.

Blood collection schedule for the assessment of ovarian steroids

Blood sample collection was started from the day zero of the natural estrus, then on every third day till 12th day of the estrus. On day 13th of natural estrus prior to buFSH and $PGF_{2\alpha}$ administration then prior to the administration of buLH, then on every day till day 0 of the next estrus. Laparotomy was performed within 24 hrs (Within five days Post PG treatment, if heat was not detectable) of the onset of the estrus Post treatment. After laparotomy ovaries were examined for macroscopic response.

With some modifications in the method of Hall and Safi (1981), radioimmunoassay was done for the ovarian sex steroids viz., estradiol and progesterone.

RESULTS

Effect on the status of the ovarian activity

The extent of appearance of follicles and corpora lutea as observed following the treatment with buFSH+buLH to goats are presented in table 1.

These presentations indicate that the total number of follicles have been increased (12.66) in the treated group as compared to that with control goats (8.50), but the percentage of the follicles above 2 nm diameter got reduced (23.74%) following the buFSH+buLH treatment in comparison with the parallel control group (51.48%). Again significantly higher number (2.33 C. L./goat) of corpora lutea were shown by the treated goats in comparison to those shown by the control group (1 C. L./goat). Visual observations recorded at laparotomy in the

Table 1. Effect of administration of buFSH with buLH on the ovarian structures observed at laparotomy

Sl No.	Control Group				Treated Group			
	Total No. of follicles (in both ovaries)	Follicles more than 2 mm diameter		No. of C. L.	Total No. of folliceles (in both ovaries)	Follicles more than 2mm diameter		No. of C. L.
		No.	%			No.	%	
1	7	4	57.14	1	–	–	–	–
2	7	4	57.14	–	13	2	15.38	2
3	12	5	41.66	–	14	4	20.57	3
4	8	4	50.00	1	11	3	27.27	2
Mean ± S. E. M	8.50 ±2.06	4.25 ±0.43	51.48 ±6.37	1	12.66 ±1.24	3.00 ±0.81	23.74 ±5.93	2.33 ±0.47

control and buFSH + buLH treated group are presented in figure 1 to 3.

Figure 1. Control group ovary.

Figure 2. buFSH + buLH Treated ovary.

Figure 3. Treated ovary showing corpora lutea.

Effect on the profile of the ovarian hormones

Data on the estradiol - 17 β profile exhibited by the buFSH + buLH treated and concurrent control group of goats in presented in table 2 and figure 4.

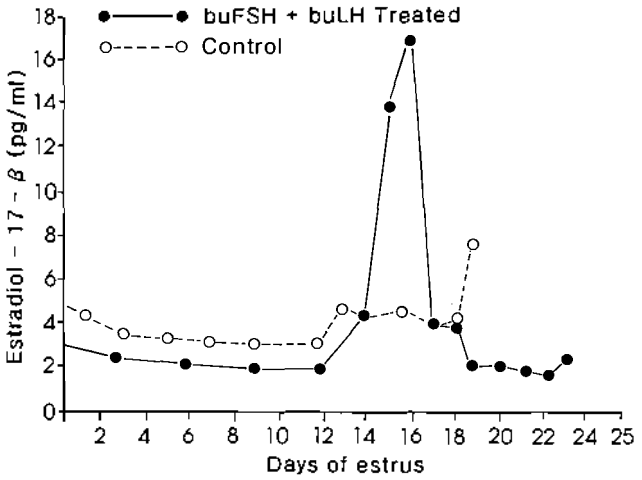

Figure 4. Effect of buFSH + buLH on the plasma estradiol 17 - β profile.

Table 2. Effect of buFSH with buLH treatment on plasma estradiol - 17 β profile

Group	Sl. No.	Range of E_2 concentration (pg/ml)	Peak E_2 value (pg/ml)	Days exhibiting higher E_2 levels
Control	1	1.1 to 7.6	7.6	13 to 13
Group	2	3.4 to 7.0	7.0	14 to 16
	3	3.2 to 4.8	4.8	15 to 16
	4	2.2 to 9.5	9.5	19 to 19
Mean ± S. E. M		2.47 ± 0.91 to 7.22 ± 1.67	7.22 ± 1.67	15.25 ± 2.27 to 16.00 ± 2.12
buFSH ± buLH	1	1.66 to 7.66	7.66	15 to 16
treated group	2	1.66 to 26.66	26.26	16 to 16
Mean ± S. E. M		1.66 to 17.16 ± 9.52	17.16 ± 9.52	15.5 ± 0.50 to 16.0

The range of the E_2 levels in goats treated with buFSH + buLH have been widened due to the combined gonadotrophin treatment. The peak of the E_2 levels achieved by the treated group have been highly enhanced (17.16 pg/ml) as compared to the peak E_2 levels (7.22 pg/ml) of the control group.

The trend of the P_4 profile exhibited by the individual goats belonging to the control and the treated group is evident from the table 3. There is a significant widening of the range of the P_4 values recorded for the buFSH + buLH treated goats as compared to that of the control group goats. Further, higher peak values for P_4 have been achieved in the treated group than the control group. Achievement of this peak looked postponed and short lived in the treated group as compared to that in the control.

Table 3. Effect of buFSH + buLH treatment of plasma progesterone profile

Group	Sl. No.	Range of P_4 concentration (ng/ml)	Peak P_4 value (ng/ml)	Days exhibiting higher P_4 levels
Control	1	0.014 to 1.03	1.03	6 to 13
Group	2	0.044 to 0.70	0.70	6 to 13
	3	0.040 to 0.70	0.70	9 to 9
	4	0.011 to 0.48	0.48	6 to 13
Mean ± S. E. M		0.03 ± 0.014 to 0.72 ± 0.19	0.72 ± 0.19	6.75 ± 0.75 to 12 ± 1
buFSH ± buLH	1	0.032 to 1.00	1.00	9 to 14
treated group	2	0.085 to 1.00	1.00	10 to 13
Mean ± S. E. M		0.05 ± 0.026 to 1.00	1.00	9.5 ± 0.50 to 13.5 ± 0.50

DISCUSSION

Results presented for treated group indicate presence of more (12.66) total number of follicles, but the percentage of large follicles have been recorded less. It seems possible that administration of buLH following the buFSH administration speeded up the maturation and development of the larger follicles which were present at the time of buLH administration beyond the stage of ovulation reducing the population of larger follicles and leading to the formation of increased number of the corpora lutea. It seems that the combined buFSH + buLH treatment have been more effective for promoting the ovulation formation of the C. L.

There are indications from earlier workers pointing out that optimum combination of FSH together with LH (an optimum Cocktail of these two) promotes better rate of ovulation than administration of FSH alone (Chupin et al., 1985 and Scanlon, 1972). According to McDonald (1980), the more possibly the goat and the pre-pubertal animals need supplementary treatment with LH or LH like gonadotrophins after FSH treatment to induce superovulations.

Despite the presence of increase in the total number of follicles, lesser number of larger follicles have been found present following the buLH treatment, the increased E_2 level in the treated group seem in order as reported by Lemon and Saumande (1972) also. Lopez-Barbella et al. (1979) observed a relationship between plasma estrogen concentration and the number of corpora lutea. Similar observations were made in the present studies.

Jain and Madan (1986) found higher E_2 levels in the goats treated with PMSG + HCG than in the goats treated with PMSG alone. Similar observations have been recorded in the present study.

Treated goats reflected a wider range of P_4 values and also an apparent elevation of the peak P_4 values. Further, there is a postponement of the day of estrus exhibiting peak P_4 values and a shorter duration showing the higher plasma P_4 levels. These changes in the plasma profile do not look as a reflection of the buFSH treatment as much as they look due to the impact of the buFSH treatment to this group, except for the profile which is revealed on or after day 19th post buLH treatment, which reflects some rise in the plasma P_4 level along with delay in the appearance of the behavioural estrus recorded. Increased number of corpora lutea observed at laparatomy in the gonadotropin treated group fits well into these observations, although quantitatively the number of corpora lutea may not always be related to the peak P_4 level (Testart et al., 1977 and Wheaton et al., 1988).

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