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CHANGES IN TOTAL LIPIDS AND CHOLESTEROL VIZ-A-VIZ STEROIDOGENIC FUNCTIONS IN CAPRINE CORPORA LUTEA

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ABSTRACT

Biochemical variations in total lipids and cholesterol contents in relation to steroidogenesis have been analysed in seven physiologically distinct categories of corpora lutea viz small (1-5 days), medium (6-10 days),large (11-15 days), regressing (16-21 days), previous, penultimate and that of pregnancy(<30 days). Total lipid content increased from small category (4.39 ± 0.66 mg/100mg) to medium category (5.148 ± 1.87 mg/100 mg) then decreased in large category to 1.840 ± o.52 mg /100 mg and then subsequently increased during regression (3.108 ± 0.84 mg /100 mg). During pregnancy value observed was 2.707 ± 0.11 mg/100 mg. Cholesterol content also increased from small category (1.070 ± 0.043 µg/100mg) to large category (2.349 ± 0.72 µg /100 mg) decreased during large category (0.315 ± 0.04 µg /100 mg). The value of cholesterol during regression was 2.683 ± 0.31 µg/100 mg and was minimum during pregnancy 0.275 ± 0.02 µg/100 mg. Immunocytochemically progesterone positive gold particles were observed in maximum frequency during pregnancy and minimum in regressing category depicting maximum steroidogenic activity during pregnancy.

Keywords: Corpus Luteum, Goat Ovary, Lipids, Cholesterol, Labelled Progesterone

INTRODUCTION

The corpus luteum, a dynamic endocrine gland, is histologically comprised of steroidogenic and nonsteroidogenic cells. The steroidogenic cells are associated with the production of steroid hormones principally progesterone for the maintenance of pregnancy **[1]**. The corpus luteum of small and large ruminants show cyclic changes in its activity **[1, 2]**. As the estrous cycle proceeds, the corpus luteum differentiates, grows in size, regresses and undergoes lysis **[3, 4]**. During luteal regression there is an increase in lipids, vacuolization and lysis of smooth endoplasmic reticulum and decline in progesterone secretion. The steroidogenic enzymes i.e. Cytochrome P450 side chain cleavage (P450 Scc), 3β hydroxy steroid dehydrogenase (3β-HSDH)/Δ5, Δ4, isomerase are involved in the production of progesterone **[5-7]**. The relationship between cholesterol content and steroidogenic activity during different stages of corpus luteum of goat have been validated in the present investigation.

MATERIALS AND METHODS

Goat (*Capra hircus*) ovaries were procured from the slaughterhouse of Delhi and brought to the Laboratory in ice bucket at 0°C. On the basis of its morphology, corpora lutea of all the seven categories **(Table1)** were dissected out, weighed and stored at 0°C for biochemical analysis.

Table 1: Classification of corpus luteum

S. No.	Size (mm) and colour	Name of category		Stage
1.	<2 Pink with red blood clot	Small	I	1-5days
2.	2-5 Pink	Medium	II	6-10days
3.	>5 Red	Large	III	11-15days
4.	2-4 Brown	Regressing	IV	16-21days
5.	≈2 Yellow	Previous	V	Pr
6.	<2 White	Penultimate	VI	Pn
7.	>6 Dark red	Pregnancy	VII	<30days

Extraction and Estimation of Total Lipids [8]

A known weight of corpora lutea was crushed in a pestle mortar in the presence of 5 grams of anhydrous sodium sulphate till a homogenous powder was obtained. The total lipids were extracted from the powder by using chloroform: methanol (2:1, v/v) mixture in the ratio of 1:20 (w/v) by intermittent shaking for at least 12 hours. The extract was filtered through G-3 sintered funnel. The residue was washed with chloroform: methanol mixture for complete extraction of lipids. The combined filtrate was taken in a separating funnel. To this combined filtrate, a folch washing with 0.9 percent saline solution

(5:1,v/v) was given. The separating funnel was kept overnight for complete separation of lipids. From the chloroform: methanol mixture, the total lipids were obtained by taking the lower fraction in a crucible of known weight, the chloroform and methanol evaporates leaving lipids in the crucible. The difference in initial and final weights of crucible gave the quantity of total lipid content. The total lipids were then dissolved in 3 ml of chloroform and stored at 4^0C and were used for quantitative analysis.

Determination of Total Cholesterol

The method of Stadman **[9]** was used for the estimation of total cholesterol. 0.5 ml of the lipid solution was taken in a test tube and to this, chloroform was added to make the total volume 5 ml. After that 1.0 ml of acetic anhydride was added and then 0.1 ml of concentrated sulphuric acid was added. After mixing the contents, the test tubes were kept at 16–18°C in water bath and allowed the colour to develop in dark for 15 minutes. The absorbance was read at 625 nm against reagent blank. A standard curve was prepared by taking cholesterol in the range of 10–80 μg/ml.

Immuno-Cytological Localization of Progesterone

For immunoelectron microscopic studies, corpus luteum was fixed in 0.5% gluteraldehyde and 2% paraformaldehyde for 8-12 hours at 4°C. The tissue was processed according to Dasgupta *et al*., **[10]**. The sections of 60-80 nm were cut using ultra microtome. The grids were put in 2% skimmed milk for 30 minutes and then immersed in 1:100 antiprogesterone primary antibodies for 2 hours at room temperature. 1° antibody were raised in Soviet Chinchilla rabbit by injecting 300μl of the immunogen (prepared by dissolving progesterone-α—OH hemisuccinate-BSA in phosphate buffer saline and emulsified with an equal volume of Freund's Complete Adjuvant). After immunization blood samples were collected and allowed to clot at room temperature and serum was separated by centrifugation. Serial dilutions of antiserum were made to estimate the titre by direct radioimmunoassay method of Kamboj and Prakash **[11]**. Secondary labelling was done for 2 hours at room temperature. After washing with phosphate buffer and distilled water, these were stained in uranyl acetate followed by lead citrate. The sections were examined and photographed under electron microscope at 60-80 KV using Morgagni 268D TEM from FEI, Netherland, installed at All India institute of Medical Sciences, New Delhi.

Statistical Analysis

The under mentioned statistical formulae were employed for statistical analysis of the result obtained during the present investigation **[12]**.

1. Mean and Standard deviation
2. Student "t" test

Formula to calculate 't' test

$$t = (\bar{x}_1 - \bar{x}_2) / s \; x \sqrt{n_1 n_2 / n_1 + n_2}$$

$$s = \sqrt{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_1 + n_2 - 2)}$$

where;

$\bar{x}_1 - \bar{x}_2 =$ **Difference of mean of sample 1 and sample 2**

n_1 and n_2 = Number of observations of sample 1 and sample 2.

S= combined standard deviation of two samples

S_1 and S_2 = standard deviation of sample 1 and sample2

RESULTS AND DISCUSSION

The total lipid content was estimated in different categories of corpus luteum (Table 2). The minimum total lipid content was recorded in large category (1.840 ± 0.52 mg/100 mg wt. of the tissue) while the maximum content was observed in medium category (5.148 ± 1.87 mg/100 mg wt. of the tissue) **(Figure 1)**. The cholesterol content of different categories of corpus luteum is given in **Table 2**. The minimum amount was recorded in corpus luteum of pregnancy (0.275 ± 0.02 μg/100 mg) and the maximum content was observed in regressing category (2.683 ± 0.31 μg/100 mg) **(Figure 1)**.

Immunocytological Localization of Progesterone Harmone

Immunocytological localization of progesterone harmone in corpus luteum of small (1-5 days), regressing (16-21 days) and that of pregnancy (<30 days) categories of goat were carried out using gold labelled **(Table 3, Figures 2, 3, 4)**. The maximum number of immunolabelled progesterone positive gold particles (5.06/cm^2) were observed in corpus luteum of pregnancy and minimum number of immunolabelled progesterone positive gold particles (1.68/cm^2) were observed in corpus luteum of regressing category (16-21 days).

In goat corpus luteum total lipids and cholesterol were minimum on day 11-15 of large category and subsequently increased during regressing category (16-21 days). In bovine corpus luteum very little lipids were observed on day 10 to 13, their amount increased during regression **[13-16]**.

The quantitative analysis of the lipids revealed a similar trend in buffalo, cow, ovine and goat **[17-19]**. Histochemical analysis of the corpus luteum revealed a similar pattern of changes in sudanophilic lipids in granulosa

luteal cells of goat. These changes have been related it to the progesterone synthesis and secretion **[19]**. During the luteal phase and early pregnancy the small luteal cells showed minimum to less frequent number of lipid droplets, this may be because the cholesterol and its esters are utilizd during active synthesis of progesterone **[20]**. The increase in the lipid droplets and vesicles during later phase of the estrous cycle is because of poor mobilization of lipids, and decline in progesterone synthesis **[14, 15, 19, 21]**. These changes correspond to the variations in progesterone particles during estrous cycle.

During pregnancy development of diffuse lipoproteins is correlated to the highest rates of conversion of acetate–1–14C into progesterone *in vitro* **[14, 22-24]**. The correlation of morphological and biochemical studies has revealed that membranes of agranular endoplasmic reticulum (or diffuse lipoproteins) play a major role as sites for enzymes that are involved in steroidogenesis **[5, 25-28]**. During regression, the luteal cells also showed increase in lipids and cholesterol similar trend to highly sudanophillic lipids as observed earlier by Guraya **[15]**. Similar pattern of changes have been reported in sheep wherein such changes were recorded from day 15 onwards **[13]**. These authors have further reported that there is a decline in 3β-hydroxy steroid dehydrogenase (3β-HSDH) and diphosphorase enzyme activity which may be responsible for elevation in lipid content. The progressive increase in cholesterol containing lipid droplets closely corresponds to the decreased level of progesterone **[5, 24, 29]**. The variations observed in the gold particles in luteal cells of different categories of goat corpora lutea are inversely correlated with the total lipids and cholesterol.

Biochemistry of the corpus luteum also revealed conspicuous changes in total lipid content and cholesterol during involution and luteal regression. Lipids are actively involved in many metabolic pathways and cholesterol in particular shows variations corresponding to the progesterone synthesis **[18, 30-34]**. Immunocytochemical studies carried on the corpus luteum of goat further endores this concept **[30-32, 35]**.

Table 2: Variations in the Amount of Total Lipids and Cholesterol of Different Categories of Corpus Luteum of Goat

Categories of Corpus Luteum	Biochemical Parameters	
	Total lipid (mg/100mg)	Cholesterol (μg/100mg)
I Small (1-5 days)	4.398 ± 0.66 (3.521-5.128)	1.070 ± 0.43 (0.640-1.500)
II Medium (6-10 days)	5.148 ± 1.87 (2.641-7.142)	2.349 ± 0.72 (1.333– 2.914)
III Large (11-15days)	1.840 ± 0.52 (1.315-2.365)	.315 ± 0.04 (0.266 -0.363)
IV Regressing (16-21 days)	3.108 ± 0.84 (2.173-4.210)	2.683 ± 0.31 (2.250–3.000)
V Previous (Pr)	2.615 ± 0.21 (2.400-2.830)	1.133 ± 0.20 (0.933-1.333)
VI Penultimate (Pn)	2.240 ± 0.06 (2.173-2.307)	0.733 ± 0.07 (0.666-0.800)
VII Pregnancy (< 30 days)	2.707 ± 0.11 (2.600-2.815)	0.275 ± 0.02 (0.260-0.290)

(Range in Parenthesis)

Table 3: The Number of Gold Particles Observed in Different Categories of Steroidogenic Cells of Corpus Luteum of Various Categories

Cell Types	Small Category (1-5 days)	Regressing Category (16-21 days)	Pregnancy (<30 days)
Small theca luteal cells	2.64 (29)	1.10 (12)	6.0 (65)
Large granulosa luteal cells	3.00 (33)	2.00 (22)	3.45(38)

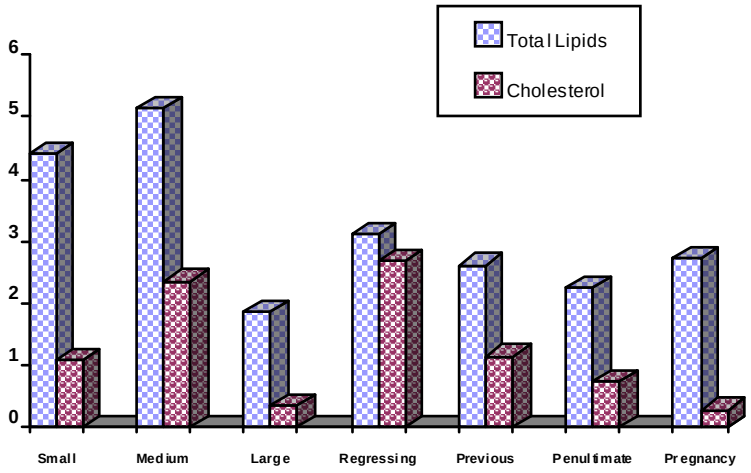

Figure 1: Changes in the Amount of Total Lipids (mg/100mg) and Cholesterol (μg/100mg) in Different Categories of Corpus Luteum of Goat

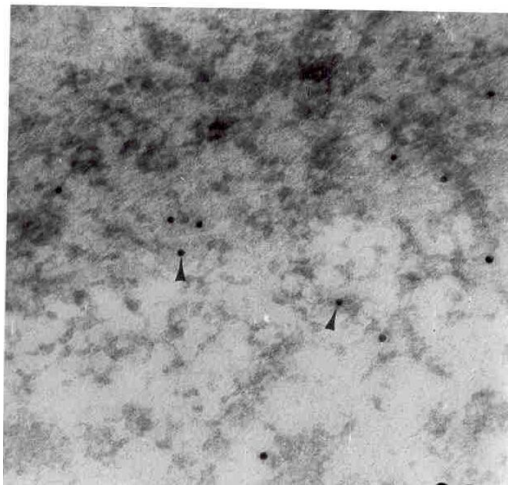

Figure 2: Immono Electronmicrograph of Granulosa Luteal Cell Showing the Presence of Progesterone Positive Gold Labelled Particles (3.00/cm^2) in Corpus Luteum of Small Category (1-5 days); X 100800

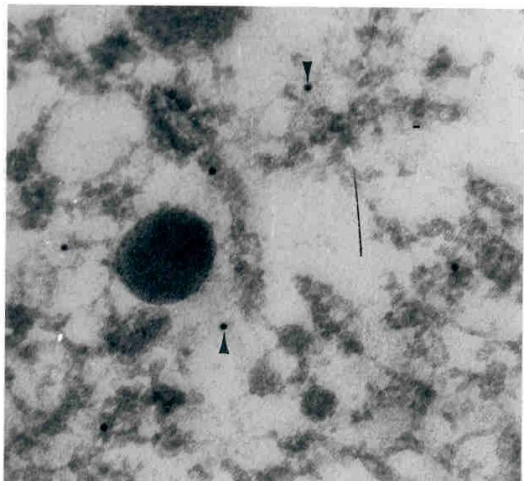

Figure 3: Granulosa Luteal Cell From Corpus Luteum of Regressing Category (16 – 21 days) Showing the Number of Gold Particles (2.00/cm^2); X 100800

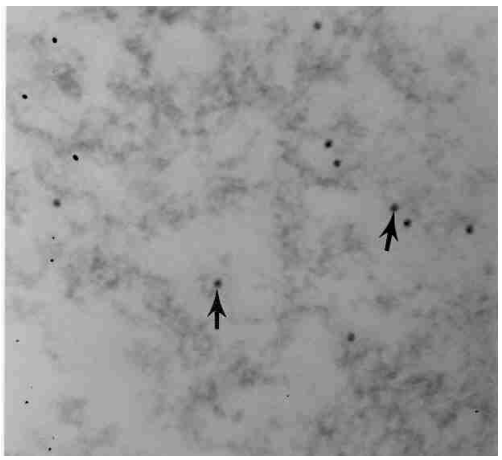

Figure 4: A High Power View of Corpus Luteum of Pregnancy Revealing the Distribution of Progesterone Positive Gold Particles (3.45/cm^2); X 126000

CONCLUSIONS

The maximum number of immunolabelled progesterone positive gold particles observed in corpus luteum of pregnancy (< 30 days) is an index of cholesterol substrate depletion for steroidogenesis. The minimum level of lipids and cholesterol are related to enhanced number of immunolabelled progesterone particles depicting maximum harmone output.

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REFERENCES

[1] Fields MJ, Fields PA, Morphological characteristics of the bovine corpus luteum during the estrous cycle and pregnancy, Therio., 45, 1996, 1295-1326.

[2] Sharma RK, Structural analysis of cumulus and corona cells of goat antral follicles: Possible functional significance, Indian J. Anim. Sci., 73 (1), 2003, 28-32.

[3] Weber DM, Fields PA, Romrell LJ, Tumwasorn S, Ball BA, Drost M and Fields MJ, Functional differences between small and large luteal cells of the late-pregnant Vs. non-pregnant cow, Biol. Reprod., 37, 1987, 685-698.

[4] Berisha B and Schams D, Ovarian function in ruminants, Domest. Anim. Endocrinol., 29, 2005, 305-317.

[5] Logan KA, Juengel JL and Mc Natty KP, Onset of steroidogenic enzyme gene expression during Ovarian follicular development in sheep, Biol Reprod., 66 (4), 2002, 906-916.

[6] Devoto L, Kohen P, Vega M, Castro O, Gonzalez RR, Retamales I, Carvallo P, Christenson LK and Strauss JF III, Control of human luteal steroidogenesis, Mol. Cell Endocrinol., 186, 2002, 137-141.

[7] Tomac J, Cekinovic D and Arapovic J, Biology of corpus luteum, Period biol., 113(1), 2011, 43-49.

[8] Folch I, Lees M, Sloanestantley GH, A simplified method for the isolation and purification of total lipids from animal tissue, J. Biol. Chem., 1957, 226, 497.

[9] Stadman ER, In: Methods of Enzymology (Ed.) Newfeld, E.T. Vol. III. Academic Press, New York, 1957.

[10] Dasgupta N, Kapur V, Singh KK, Das TK, Sachdeva S, Jyothisri K, and

Tyagi JS, Tubercle Lung Dis., 80 (3), 2000, 141-159.

[11] Kamboj M and Parkash BS, Tropical Animal Health and Production, 25, 1993, 185-192.

[12] Zar JH, Biostatistical Analysis, Prentice – Hall Inc. Englehood Cliffs., NJ, 1984.

[13] Deane HW, Hay MF, Moor RM, Rowson LEA and Short RV, The corpus luteum of the sheep: relationship between morphology and function during the oestrus cycle, Acta Endocrinol., 51, 1966, 245-263.

[14] Guraya SS, Ovarian Biology in Buffaloes and Cattle, Directorate of Information and Publications of Agriculture ICAR, New Delhi, 110012, 1997b.

[15] Guraya SS, Comparative Cellular and Molecular Biology of Ovary in Mammals: Fundamental and Applied Aspects, Oxford and IBH Publishing Co. Pvt. Ltd., India, 2000.

[16] Sangha GK, Sharma RK and Guraya SS, Biology of corpus luteum in small ruminants, Small Ruminant Research, 2002, 43, 53-64.

[17] Sharma RK, Vats R and Sawhney AK, Biochemical changes in lipids during follicular growth in goat, *(Capra hircus)*, Small Ruminant Res., 20, 1996b, 177-180.

[18] Waterman RA, Changes in lipid contents and fatty acid composition in ovine corpora lutea during the estrous cycle and early pregnancy, Biol. Reprod., 38, 1988, 605-615.

[19] Brar AS, Morphological, histochemical and biochemical studies on the mammalian corpus luteum, Ph.D. Dissertation, Punjab Agricultural University, Ludhiana, India, 165, 1993.

[20] Miyamoto H, Manabe N, Ishibashi T and Utsumi K, Histochemical observations on lipids in the goat ovary, Jpn. J. Zootech. Sci., 55, 1984, 101-106.

[21] Singh GK, and Prakash P, Histomorphological and histochemical studies on the ovary of goat, Indian Vet. J, 65, 1988, 705-709.

[22] Milvae RA, Hinckley ST and Carlson JC, Luteotropic and luteolytic mechanisms in the bovine corpus luteum, Therio., 45, 1996, 1327-1350.

[23] Guraya SS, Comparative biology of corpus luteum: cellular and molecular regulatory mechanisms, In: Maitra

SK (Ed.), Frontiers in Environmental and Metabolic Endrocrinology, 1997a, 31-58.

[24] Quirke LD, Juengel JL, Tisdall DJ, Lun S, Heath DA, Mc Natty KP, Ontogeny of steroidogenesis in the fetal sheep gonad, Biol Reprod., 65 (1), 2001, 216-28.

[25] Pate JL and Condon WA, Effects of serum and lipoproteins on steroidogenesis in cultured bovine luteal cells, Molec. Cell. Endoct., 28, 1982, 551-562.

[26] Pate JL and Condon WA, Regulation of steroidogenesis and cholesterol synthesis by prostaglandin $F_2\alpha$ and lipoproteins in bovine luteal cells, J. Reprod. Fertil., 87, 1989, 439-446.

[27] O'Shahugnessy PJ and Wathes DC, Role of lipoproteins and denovb cholesterol synthesis in progesterone production by cultured bovine luteal cells, J. Reprod. Fert., 74, 1985, 425-432.

[28] Huet C, Monget P, Pisselet C, Monniaux D, Changes in extracellular matrix components and steroidogenic enzymes during growth and atresia of antral ovarian follicles in the sheep, Biol. Repord., 56 (4), 1997, 1025-1034.

[29] Guraya SS, The cellular and molecular biology of gonadal development and maturation in mammals: Fundamental and Biomedical implications, Narosa Publishing House, New Delhi, 1998.

[30] Guraya SS, Morphology, histochemistry and biochemistry of human ovarian compartments and steroid hormone synthesis, Physiol. Rev., 51, 1971, 785-807.

[31] Guraya SS, Physiology of rat corpora lutea during various reproductive states, J. Sci. Indust. Res., 35, 1976, 739-749.

[32] Guraya SS, Recent advances in the morphology, histochemistry, biochemistry and physiology of bovine ovarian compartments and steroid hormone synthesis, J. Anim. Morph. Physiol., Silver Jubilee, 1978, 86-103.

[33] Niswender GD and Nett TM, The corpus luteum and its control, In "The Physiology of Reproduction" (E. Knobil, and J. D. Neill, Eds.), pp 489-526, Raven Press, New York, 1988.

[34] Niswender GD and Nett TM, Corpus luteum and its control in intraprismate species, In: Physiology

of Reproduction, Vol.-I. 2nd Ed., (E. Krobil, and J.D. Neil, Eds.) pp. 781-816, Raven Press, New York, 1994.

[35] Ahmed T, Gupta BC, Sidhu KS and Guraya SS, Histochemical studies on the smooth, preovulatory and luteal stage buffalo (*Bubalus bubalis*) ovary, Indian J. Anim. Res., 5, 1984, 15-18.