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Lipoprotein Lipase Activity in the Bovine Corpus Luteum during the Estrous Cycle and Early Pregnancy[1] (39282)

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Shemesh and Hansel (1) have shown that the bovine ovary has the ability to produce prostaglandins of the F series *in vivo*. Addition of arachidonic acid to bovine luteal tissue *in vitro* increased prostaglandin F synthesis (2) which suggested that this tissue contained "prostaglandin synthetase" and possessed the ability to produce large amounts of PGF when provided with high concentrations of free arachidonic acid. However, it was not known whether the bovine corpus luteum had the ability to obtain free fatty acids (including arachidonic) from plasma triglycerides and phospholipids, and if the activities of the hydrolytic enzymes changed during the estrous cycle.

The present investigation was conducted to determine whether the bovine corpus luteum has the ability to derive free fatty acids from plasma lipoprotein triglycerides. Lipoprotein lipase (LPL) activity was measured in corpora lutea collected at different stages of the estrous cycle and early pregnancy and the activities were correlated with progesterone levels in these corpora lutea.

Materials and methods. Corpora lutea were removed from 23 normal Holstein heifers at accurately dated stages of the estrous cycle and early pregnancy through an incision in the anterior wall of the vagina. These corpora lutea were immediately chilled, weighed, and frozen.

Acetone-ether powders of the homogenized corpora lutea were prepared as described by Patten and Hollenberg (3). Extraction of 100-mg samples of the acetone-ether powders was accomplished with 1 ml of 1.2 *M* NaCl, 20% glycerol, 0.005 *M* sodium barbital buffer, pH 6.5 (4). LPL was determined in duplicate samples in two different incubations, using a synthetic [^{14}C]triolein substrate labeled in the oleic acid moiety. The substrate was emulsified in the presence of gum arabic, as described by Bensadoun *et al.* (4). The specific activity of the substrate was 126,000 cpm/μmole triolein.

The assay system contained the following components in a total volume of 0.5 ml: 1.25 μmole [^{14}C]triolein, 2.5 mg gum arabic, 5 mg albumin, 0.01 ml rat serum, 0.1 mmole Tris buffer, 0.05 mmole NaCl, 5 μmole $CaCl_2$, and 0.02 ml enzyme solution. Assays were conducted at pH 8.6 at 30°. Labeled free fatty acids were separated from the substrate by the liquid–liquid partition system of Belfrage and Vaughan (5). Variation in NaCl molarity (0.1 to 1.0 M) and presence or absence of serum or heparin did not affect the partition coefficient of [^{14}C]oleic. Extraction and analysis of progesterone was as described by Apgar *et al.* (6).

For the LPL determination, five or six corpora lutea each at Days 4–8, 12–15, 16–18, and 19–20 of the cycle were utilized. In addition, LPL was determined in corpora lutea removed at slaughter from two animals at the twenty-second day of pregnancy. For the progesterone determinations, three or four corpora lutea each at Days 4–8, 12–15, 16–18, and 19–20 of the estrous cycle were used, in addition to the two corpora lutea of pregnancy.

Results and discussion. Table I summarizes properties of the lipolytic activity measured. The lipolytic activity determined in bovine corpora lutea exhibits properties similar to mammalian adipose tissue lipoprotein lipase (4). The activity was inhibited 83% by 1 *M* NaCl and stimulated 139% by heparin (2 units/ml incubation medium). Enzymic activity was also serum-dependent.

The LPL activity (μmole, of fatty acid released/hr/100 mg acetone powder) was low in corpora lutea removed at Days 4–8 of the estrous cycle (3.1 ± 1.5 mean ± SE) and at Days 19–20 (1.6 ± 0.6). However,

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high enzymatic activity was found on Days 12–15 of the cycle (11.8 ± 1.8); these concentrations were significantly ($P < 0.01$) elevated over those found at Days 4–8 and 19–20 (Fig. 1). Enzymatic activity declined on Days 16–18 of the estrous cycle (5.1 ± 1.7). Low activity was found in the corpora lutea removed from the two cows on Days 22 of pregnancy.

Progesterone concentrations of the corpora lutea are also shown in Fig. 1. Progesterone concentrations increased from 10.3 ± 1.8 (mean ± SE; μg/g) at Days 4–8 to 27.4 ± 2.8 at Days 12–15 ($P < 0.05$) and remained high at Days 16–18 (26.2 ± 2.2). Low levels of progesterone were found at Days 19–20, (1.6 ± 0.3). The regression line of progesterone concentration vs the concentrations of lipoprotein lipase is shown in Fig. 2. The measurements were highly correlated ($r = 0.75$, $P < 0.01$).

TABLE I. CHARACTERIZATION OF TRIGLYCERIDE LIPASE OF BOVINE LUTEAL TISSUE.

Conditions	Enzyme activity (mean ± S.E.) (μmole fatty acids/100 mg powder/hr)
Complete system[a]	2.3 ± 0.08
+ heparin (2 U/ml)	5.5 ± 0.31
− serum	ND[b]
+ NaCl (1 *M*)	0.4 ± 0.11

[a] The complete system contained the following components in a total volume of 0.5 ml: 1.25 μmole of [^{14}C]triolein; 2.5 mg of gum arabic; 5 mg of albumin; 0.01 ml of rat serum; 0.1 mmole of Tris buffer, pH 8.6; 0.025 mmole of NaCl; 5 μmole of $CaCl_2$; and 0.02 ml of enzyme solution.

The enzyme solution was obtained by extracting 100 mg of acetone powder with 1 ml of buffered saline described in the Methods section. Each value represents mean ± SE of three observations.

[b] ND = not detectable: Activity was lower than 0.05 μmole fatty acid/100 mg acetone powder/hr.

Benson *et al.* (7) recently reported the presence of an ovarian LPL associated with the granulosa cells of the hen. These authors postulated that this lipolytic activity might mediate the transfer of plasma triglyceride fatty acid to the oocyte.

The present results demonstrate for the first time that LPL is present in the bovine corpus luteum and that high activity of the enzyme is found at Days 12–15 of the cycle, compared with that found at other days of the cycle and early pregnancy (Fig. 1). The fact that high activity of the enzyme was present in the corpus luteum 4 days before regression and that low activity of the enzyme was found in early pregnancy suggests that LPL may be involved in the luteolytic process. The high levels of LPL at Days 12–15 may initiate the luteolytic processes by

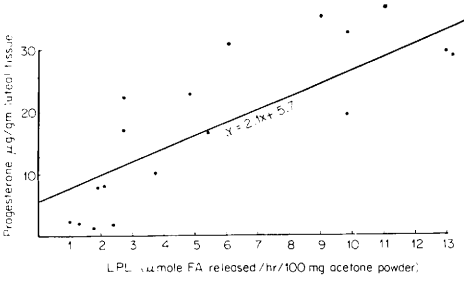

FIG. 2. Comparison between bovine corpus luteum progesterone concentration and lipoprotein lipase activity.

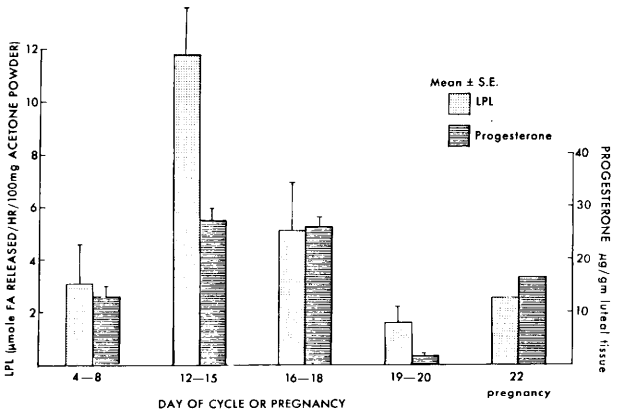

FIG. 1. Concentrations of progesterone and lipoprotein lipase in the bovine corpus luteum at different stages of the estrous cycle and early pregnancy.

increasing concentrations of free arachidonic acid supplied to the luteal tissue from plasma lipoproteins. Precursor concentration appears to be a major factor limiting PGF biosynthesis in bovine luteal tissue (1, 2). A similar mechanism may exist in the rat, since lipoprotein lipase activity has been found to increase eightfold in ovarian homogenates from pregnant rats two days before parturition (8).

A good correlation ($r = 0.75$, $P < 0.01$) was found between LPL and progesterone concentrations in the tissue. The fact that LPL appears to decline at Days 16–18, prior to the decrease in progesterone concentration, may be due to the relatively low number of observations made at that time. The progesterone concentration at Days 16–18 (Fig. 1) represents a mean of only three determinations.

Summary. Lipolytic activity measured at pH 8.6 in bovine corpora lutea exhibited classical properties of lipoprotein lipase (LPL) in terms of serum and heparin stimulation and NaCl inhibition. LPL activity was measured in 23 corpora lutea collected at different stages of the estrous cycle and early pregnancy. The LPL activity in cyclic corpora lutea (μmole FA released/hr/100 mg acetone powder) was low at Days 4–8 of the estrous cycle (3.1 ± 1.5: mean ± SE) and at Days 19–20 (1.6 ± 0.6). However, high activity of the enzyme was found at Days 12–15 of the cycle (11.8 ± 1.8); these concentrations were significantly ($P < 0.01$) elevated over those found at Days 4–8 and 19–20. The enzyme activity began to decline at Days 16–18 of the estrous cycle (5.1 ± 1.7). Low enzyme activity was found in the corpora lutea removed from two cows at Day 22 of pregnancy.

Progesterone concentrations were measured in 16 of the 23 corpora lutea and a good correlation ($r = 0.75$, $P < 0.01$) was found between lipoprotein lipase and progesterone concentrations of the tissue. The data suggest that LPL may be involved in controlling the transfer of fatty acids, including arachidonic, from plasma lipoproteins to luteal tissue.

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