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Cyclic Fluctuations of Plasma Cholesterol in the Female Miniature Swine and Its Relationship to Progesterone Secretion (39696)[1]

S. LUSSIER-CACAN, E. BOLTE, M. BIDALLIER, Y. S. HUANG, AND J. DAVIGNON

Department of Lipid Metabolism and Atherosclerosis Research, Clinical Research Institute of Montreal, 110 Pine Ave. West, Montreal, Quebec H2W 1R7; Section of Vascular Medicine and Service of Endocrinology, Hôtel-Dieu Hospital, Montreal, Quebec H2W 1T8; and University of Montreal, Faculty of Medicine, CP 6128, Montreal, Quebec H3C 3J7, Canada

There have been few studies on the influence of the estrous cycle on blood lipid concentrations in animals. In one report, the total plasma cholesterol measured during the rat estrous cycle was found to be higher during proestrus and estrus than during diestrus (1). In another study (2), blood was obtained from mature ewes on the day of estrus and 6 days later during diestrus. Serum cholesterol concentrations tended to be lower during diestrus, but the difference between the two phases was not statistically significant. To our knowledge this information is not available for porcine animals, which are often used as models for the study of atherosclerosis.

During our studies on the effect of portacaval anastomosis on cholesterol metabolism in the female miniature swine, we were confronted in some animals with the problem of excessive variation in plasma cholesterol. Changes as large as 50% were observed for some animals during the preoperative phase, with values in the range of 130–155 mg/dl, considered inordinately high compared to the 80–100 mg/dl usually found and expected for normal pigs in the age range studied (3). This phenomenon became even more serious when it reappeared in the postoperative phase since it seriously compromised evaluation of the effect of the shunt on plasma lipids. Daily or frequent blood sampling over long periods showed that the fluctuation was cyclic and led us to suspect an effect of the estrous cycle, which was confirmed in the results to be reported.

Materials and methods. Six noncastrated female miniature pigs (Sinclair Research Farm, University of Missouri, Columbia, Missouri) aged 8–10 months were housed indoors and fed a standard pig grower mash. Four animals underwent a portacaval anastomosis, whereas two were sham-operated with surgical manipulations which included vascular clamping.

Total plasma cholesterol and progesterone were measured on samples obtained in the morning during long-term studies started 5 weeks after the operations and continuing over a total of 13 presumptive estrous cycles. Cholesterol was measured on a Technicon Autoanalyzer by the method of Block *et al.* (4). Progesterone was determined by radioimmunoassay by the method of DeVilla *et al.* (5). The coefficient of variation for our method for cholesterol assay (replicate determinations) is less than 3%.

Results. The plasma progesterone concentration was only 0.1–1.5 ng/ml during an estrogenic phase averaging 7 days, then rose rapidly to high values (15–50 ng/ml) and declined again over the next 14 days. This indicated an estrous cycle of 21 days, in agreement with previous reports (6, 7). All six animals demonstrated ovulatory cycles (Fig. 1). Plasma cholesterol varied inversely with progesterone in five of the six animals (Fig. 1). High cholesterol levels were evident for 3–6 days during the follicular phase; the peak value was usually attained 2 or 3 days before the estimated ovulation day. The latter was assumed to correspond to or immediately follow the rise in plasma progesterone. The sixth (sham-operated) animal showed normal progesterone secretion but gave no predictable cyclic pattern for cholesterol. This animal had had one anovulatory cycle prior to surgery.

The average increase in cholesterol con-

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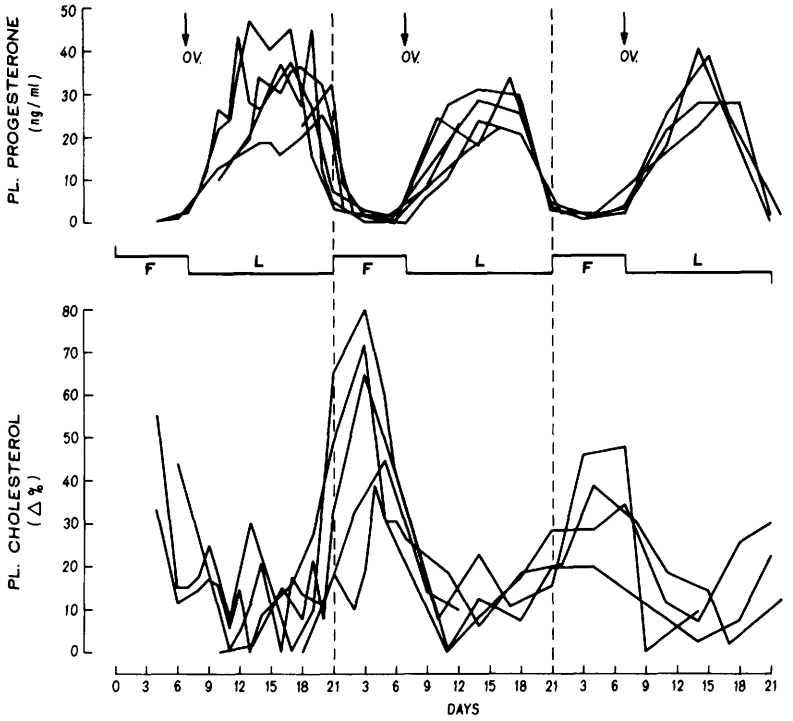

FIG. 1. Inverse cyclic relationship between plasma concentrations of progesterone (ng/ml) and cholesterol (percentage of increases over the lowest value in the luteal phase) during 21-day estrous cycles. F and L refer to follicular and luteal phases, OV. to approximate time of ovulation estimated from time of onset of the increase in plasma progesterone.

centrations at the peak was 50% of the minimum value during the progesterone secretion period. The cyclic changes persisted throughout the study and were similar for shunted and sham-operated animals (Fig. 2). However, the variations in plasma cholesterol were of a lesser amplitude in one shunted animal (No. 26, Fig. 2), whose condition deteriorated 4 months after surgery (extensive liver damage being noted at autopsy), while the progesterone secretory pattern remained unchanged.

To document this investigation further, progesterone was also measured in five plasma samples obtained from three different animals preoperatively, for which inappropriately high levels of cholesterol were observed. Progesterone levels were found to be low in every case (0.2–1.1 ng/ml).

Discussion. We do not know how sex hormones influence plasma cholesterol in the miniature swine. In their study of cholesterol and bile acid turnover in female miniature swine, Dupont *et al.* (8) found great

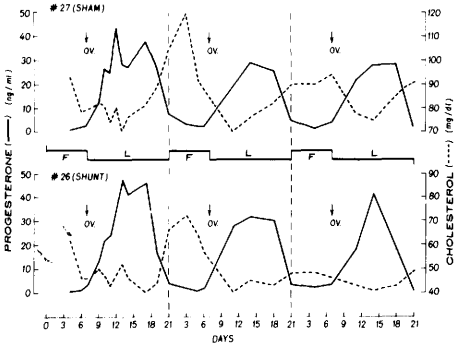

FIG. 2. Similar cyclic fluctuations for progesterone and cholesterol in the plasma of sham-operated (above) and shunted (below) single miniature swine. F, L, and OV. as in Fig. 1.

variation from sample to sample but thought this might be attributed to frequent sampling and to the effect of digestion, since the bleeding was done postprandially. Wolfe and Belbeck (9) found that the turnover of triglyceride fatty acids was increased in a female swine which was reportedly in heat,

as confirmed by serum estrogen assay. Their report did not refer to plasma cholesterol.

Several blood lipid concentrations have been shown to be affected during the hormonal changes occurring during the human menstrual cycle, but observations have been variable. Some authors have reported an increase in cholesterol at the time of ovulation (10, 1), others (11) a decrease, whereas in some studies no differences were found (12, 13). Whenever changes were observed, they were usually small and, thus, could readily be missed unless blood was sampled daily. In one study (14), lipid concentrations were measured simultaneously with urinary excretion of estrogens, pregnanediol, and several other steroids. While no correlation was found between plasma cholesterol and urinary hormone concentrations, an increase in total cholesterol during the follicular phase and a decrease during the luteal period were observed. Simultaneous determinations of plasma cholesterol and plasma gonadal steroids have so far not been reported in humans.

While increases in plasma cholesterol and especially triglycerides are well documented in human pregnancy (15, 16), alterations of plasma lipids in pregnant animals vary greatly according to species. Increased cholesterol levels occur in the rat (17) and the dog (18), whereas, in the rabbit (19) and the rhesus monkey (20), a continuous drop in plasma cholesterol is observed as pregnancy progresses. There has been, so far, no report on this phenomenon in the swine.

Our results show an evident inverse relationship between total plasma cholesterol and progesterone levels. The cholesterol peaks attained during the follicular part of the cycle are often sharp and short-lived and do not correspond to the estimated day of ovulation. Thus, random occasional blood sampling could easily cause these changes to go unnoticed. To our knowledge, this is the first report of a cholesterol fluctuation patterned on the estrous cycle in the swine. Its extent is such that blood sampling 2–3 days before ovulation may yield, in extreme cases, plasma cholesterol values 80% higher than those obtained during the luteal phase (Fig. 1).

In view of the growing popularity of miniature swine as an experimental model for the study of lipid and cholesterol metabolism (8, 9, 21–23) and because of the frequent choice of females because of their mild disposition, one should be aware of this physiological phenomenon. Failure to take it into account could seriously compromise the results of such experiments and lead to incorrect interpretation.

Summary. Large fluctuations in plasma cholesterol concentration were noted during a study on the metabolic effects of portacaval anastomosis in the female miniature swine. The fluctuations were cyclic and related to the estrous cycle, as shown by measurements of total plasma cholesterol and progesterone on samples obtained almost daily from six animals over several estrous cycles. Hormone concentrations indicated a 21-day estrous cycle consisting of a 7-day follicular phase and a 14-day luteal period. Plasma cholesterol fluctuated in a cycle which was the inverse of that for progesterone: High cholesterol concentrations were observed for 3–6 days during the follicular phase with peak values as much as 80%, and on the average 50%, higher than the mean levels observed during the luteal phase. Failure to recognize these plasma cholesterol cyclic fluctuations can totally confuse the interpretation of studies on cholesterol metabolism in swine, which are increasingly popular as experimental models of atherosclerosis and lipid metabolism.

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