Hormone changes during the menstrual cycle in abetalipoproteinemia: Reduced luteal phase progesterone in a patient with homozygous hypobetalipoproteinemia

(low density lipoprotein/steroid hormone synthesis/corpus luteum)

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ABSTRACT   Progesterone synthesis by the human corpus luteum requires a source of cholesterol, which can be derived from both local synthesis and uptake of low density lipoproteins (LDL). When the corpus luteum is maintained in organ culture, progesterone synthesis is primarily dependent on LDL and the rate of progesterone production during growth in a LDL-free media is suboptimal. An *in vivo* situation analogous to that of corpus luteum grown in LDL-depleted media exists naturally in patients with abetalipoproteinemia. To determine whether a complete deficiency of plasma LDL affects serum concentrations of progesterone (particularly during the luteal phase) or those of other hormones, we have measured the serum concentrations of luteinizing hormone, follicle-stimulating hormone, prolactin, estradiol, estrone, and progesterone during the menstrual cycle in a patient with phenotypic abetalipoproteinemia (on the basis of homozygous hypobetalipoproteinemia). Our results show a normal cyclical pattern with midcycle increases in the concentrations of luteinizing and follicle-stimulating hormones, prolactin, and estrogens but a distinctly subnormal increase in the luteal phase concentrations of progesterone. These results suggest that, in patients with phenotypic abetalipoproteinemia, the absence of LDL leads to an impairment in the maximal rates of production of progesterone by the corpus luteum.

The biosynthesis of steroid hormones is dependent on a source of cholesterol, which may be derived from both *de novo* synthesis and uptake of plasma lipoproteins (1, 2). Specific high-affinity receptors for low density lipoprotein (LDL) have been demonstrated in steroid hormone-producing tissues from bovine (3, 4), mouse (5), and human (6–9) sources and appear to be identical to those that are genetically absent in patients with receptor-negative homozygous familial hypercholesterolemia (10). Evidence that favors an important physiological role for these receptors and plasma LDL in steroid hormone synthesis has been provided from several sources. LDL receptor activity in the human is 10–20 times higher in membrane preparations obtained from adrenal or gonadal tissue as compared with those obtained from heart or lung tissue (1). Cultured human fetal adrenal cells (2, 6), trophoblastic cells (9), and choriocarcinoma (8, 11) have also been shown to specifically utilize LDL as the major source of cholesterol for steroid hormone synthesis. Recent studies suggest this is also true for the human corpus luteum (12, 13). Finally, tissue concentrations of intravenously injected [^{14}C]sucrose-labeled LDL have been shown to be 5–20 times greater in the adrenal glands of swine than in other tissues (14). Because [^{14}C]sucrose is not degraded within lysosomes,

this method provides a reliable estimate of the relative rates of uptake of labeled LDL that occur in different animal tissues *in vivo*. The rat appears to be atypical and tissues from this animal preferentially utilize high density lipoprotein as a source of cholesterol for steroid hormone biosynthesis (15, 16).

Recent studies (17, 18) in patients with phenotypic abetalipoproteinemia, a rare hereditary disorder characterized by the complete absence of all apoprotein B-containing lipoproteins (chylomicrons, very low density lipoprotein and LDL) from plasma (19, 20), have provided physiological evidence that LDL is an important source of cholesterol for adrenal corticosteroid synthesis under conditions of prolonged stimulation with corticotropin. In these patients, basal adrenal cortical function was found to be normal but the response to corticotropin stimulation was impaired (17, 18).

The human corpus luteum is, on a gram-for-gram basis, the most active steroidogenic tissue in humans. As discussed by Carr *et al.* (12), this tissue secretes 30–40 mg of progesterone daily in the midluteal phase of the menstrual cycle. If progesterone synthesis by the human corpus luteum *in vivo* is as dependent on LDL cholesterol as the *in vitro* studies suggest (12, 13), then progesterone production during the luteal phase of the menstrual cycle should be impaired in abetalipoproteinemia. The present study addresses this question and describes hormone changes during the menstrual cycle in a young woman with phenotypic abetalipoproteinemia on the basis of homozygous hypobetalipoproteinemia. Our results reveal markedly reduced concentrations of progesterone during the luteal phase of the menstrual cycle and provide *in vivo* evidence that optimal rates of progesterone biosynthesis in the human corpus luteum require a source of LDL.

METHODS

Subjects. The study was conducted at the Bay Clinic, Coos Bay, Oregon, and at the Clinical Research Center, Oregon Health Sciences University. Informed consent was obtained in accordance with the policies of the Human Research Committee. The subjects consisted of a 19-year-old female patient with phenotypic abetalipoproteinemia on the basis of genotypic homozygous hypobetalipoproteinemia and a normolipidemic 24-year-old female volunteer who served as a control. The latter was used primarily to confirm the methodological validity of our assays in comparison with published data on large numbers of normal women. Although the control subject's ovarian cycle length was abnormally short (21), the measured concentrations

Abbreviations: LDL, low density lipoprotein; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

of steroid and peptide hormones in her plasma agree closely with those from large series reported in the literature. For this reason, we feel justified in drawing on previously published data that have well established the normal concentrations of steroid and peptide hormones that occur during the ovarian cycle in normal women. Detailed reports on the clinical (22) and endocrinological aspects (18) of the patient have been reported previously. She underwent menarche at age 14, has had regular menstrual periods since age 17, and is an attractive normally developed young woman (weight, 60 kg) in whom areflexia remains the only abnormality on physical examination. She has been maintained on high-dose vitamin E therapy since age 11. During the periods of study, her medications were vitamin E (Covitol; Henkel Corporation, Minneapolis, MN), 14,000 international units/day; vitamin A (Aquasol A), 13,000 interna-

tional units/day; mephyton, 5 mg once weekly; and ferrous sulfate, 300 mg once weekly.

Assays. Blood samples were obtained daily for a period of 30 days to assess hormone changes during one complete ovarian cycle and over a separate period of 6 days in one other cycle. The sample was allowed to clot and the serum was separated by centrifugation. The serum sample was then divided into two aliquots, which were frozen in sealed plastic vials and stored at $-20°C$ until hormone assays were carried out. Serum concentrations of prolactin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone, estradiol, and estrone were determined by radioimmunoassay (BIORIA, Montreal, Canada; Miles) in the core laboratory of the Clinical Research Center. Samples from the control subject and our patient with homozygous hypobetalipoproteinemia were analyzed in parallel
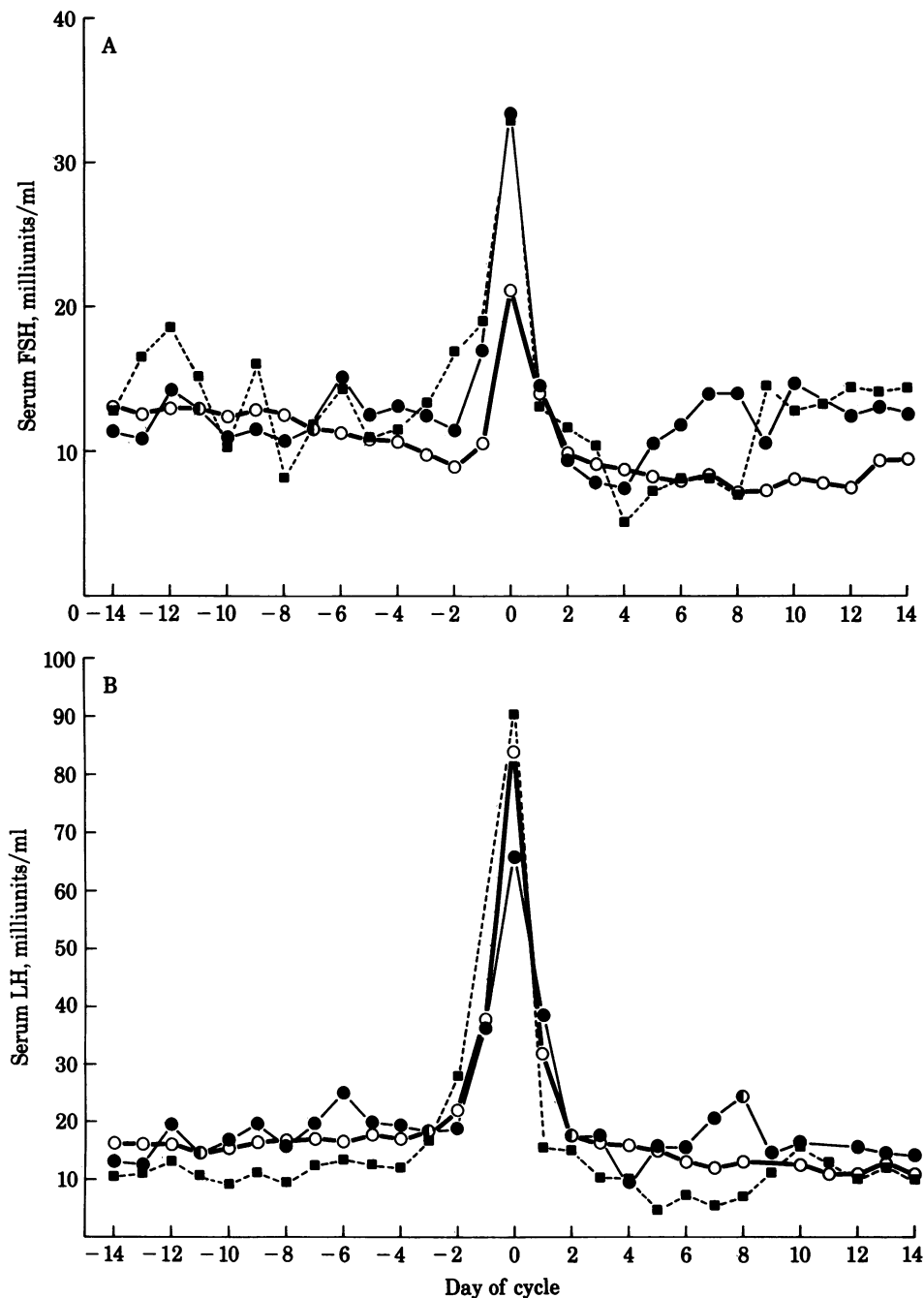

FIG. 1.   Serum concentrations of FSH (A) and LH (B) during the menstrual cycle in a patient with homozygous hypobetalipoproteinemia (●), in a normolipidemic control (■), and in normal women [redrawn from Ross *et al.* (23)] (○).

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and were stored for a similar time period (2–12 wk) prior to analysis. The 24-hr excretion of pregnanediol in the urine was determined by commercially available radioimmunoassay (Pathologists Central Labs., Portland, OR).

RESULTS

Changes in the serum concentrations of various steroid and peptide hormones that occur during the normal human menstrual cycle have been studied extensively and the normal values are well established. For this reason, we chose to study only one normolipidemic control subject and the results obtained in this subject have been used primarily to confirm the methodological validity of our assays. For comparison, the serum concentrations of hormones during the menstrual cycle in our patient with homozygous hypobetalipoproteinemia have been plotted together with those obtained in the control subject and with reported data from a large series of normal women studied by using similar assay methods.

Changes in the serum concentrations of FSH and LH in the controls (23) and our patient with homozygous hypobetalipoproteinemia are shown in Fig. 1. Both the peak and preovulatory concentrations of both hormones are normal and show that an absence of LDL does not result in any abnormalities in the serum concentrations of these hormones throughout the ovarian cycle. The same is also true for prolactin (24). Serum concentrations of prolactin (Fig. 2) in our patient with homozygous hypobetalipoproteinemia showed a biphasic pattern with peak concentrations at midcycle and again 7 to 8 days later.

Serum concentrations of the steroid hormones estradiol (25), estrone (26), and progesterone (27) are shown in Fig. 3. Estradiol levels in the patient with homozygous hypobetalipoproteinemia showed a preovulatory increase and attained a peak concentration of 192 pg/ml at midcycle, coincident with the LH peak. Although the peak concentrations of both estradiol and estrone were lower in this patient than in the controls, they still fall within the established normal range (25, 26).

Synthesis of progesterone by the corpus luteum increases rapidly after ovulation and maximal serum concentrations of 8–10 ng/ml are attained 7 to 8 days later (Fig. 3C). In our control subject, these concentrations were attained after 4 to 5 days, after which they rapidly declined. These changes typify those of a short luteal phase (21). Changes in the serum concentrations of progesterone in the patient with homozygous hypobetalipoproteinemia are also shown in Fig. 3C. Following the midcycle peaks of LH and FSH, progesterone concentrations increased slowly for 4 days, after which the values remained comparatively stable until day 9. Maximal values during this plateau period were, however, markedly reduced and never more than 1.6 ng/ml. Progesterone levels gradually declined between days 10 and 14 and menstrual bleeding began on day 15. Although the rise in serum progesterone concentrations is clearly subnormal, the sustained elevation between days 4 and 9 is not compatible with an anovulatory menstrual cycle in which serum concentrations of progesterone do not change. The reduction in the luteal phase concentrations of progesterone observed in this complete cycle have been confirmed by analyses of progesterone levels during 5 days of a second cycle. Values in the latter increased to 1.1 ng/ml; concurrent excretion of urinary pregnanediol (1.2 mg/24 hr; normal, 2–5 mg/24 hr) and pregnanetriol (0.23 mg/24 hr; normal, 0.5–3.0 mg/24 hr) was also reduced.

DISCUSSION

Several lines of evidence (reviewed in refs. 1, 2, 10) support the view that receptor-mediated uptake of plasma LDL serves as an important source of cholesterol for nonhepatic cells in the human. The quantitative importance of LDL also appears to be greatest in endocrine glands, such as the adrenal cortex and gonads, which actively secrete steroid hormones. Recent *in vitro* studies (12, 13) have examined the role of individual lipoproteins as suppliers of cholesterol for progesterone synthesis by human corpus luteum tissue maintained in organ culture. In the studies of Carr *et al*. (12), progesterone secretion was found to be 12 times higher when the culture medium contained LDL and human choriogonadotropin, as compared with a lipoprotein-free medium containing only the gonadotropin. Addition of HDL to the medium increased progesterone secretion by 50%, but the stimulatory effect of this lipoprotein was much lower than that observed with LDL. On the basis of these observations, together with *in vitro* assessments of the activity of hydroxymethylglutaryl-CoA reductase in corpus luteum tissue, both Carr *et al*. (12) and Tureck and Strauss (13) concluded that LDL serves as the primary source of cholesterol for progesterone biosynthesis and that the rates of *de novo* cholesterol biosynthesis were inadequate to satisfy the daily needs for progesterone biosynthesis.

A situation analogous to that of corpus luteum tissue maintained in a LDL-free culture medium exists naturally in patients with phenotypic abetalipoproteinemia. Although very rare, this disorder provides a unique human model in which the physiological and metabolic consequences of LDL deficiency can be
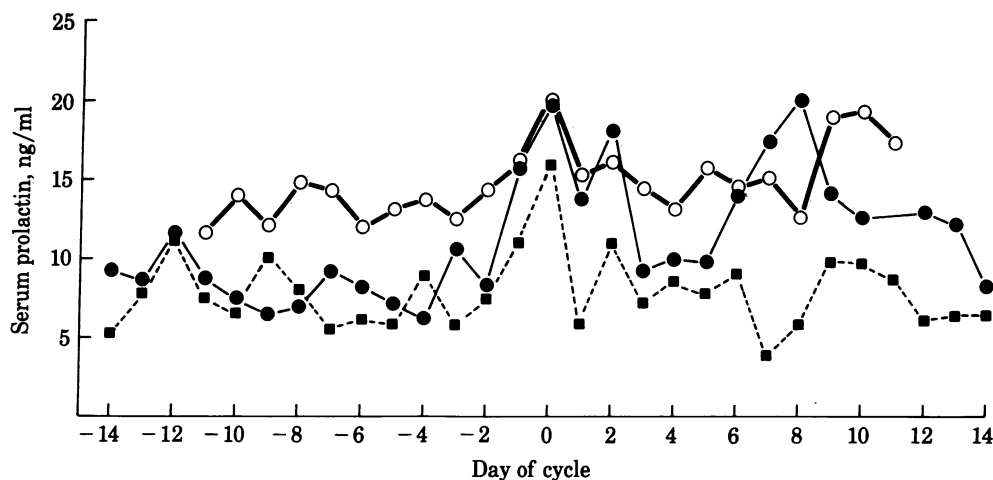

FIG. 2. Serum concentration of prolactin during the menstrual cycle in a patient with homozygous hypobetalipoproteinemia (●), in a normolipidemic control (■), and in normal women [redrawn from Vekemans *et al*. (24)] (○).
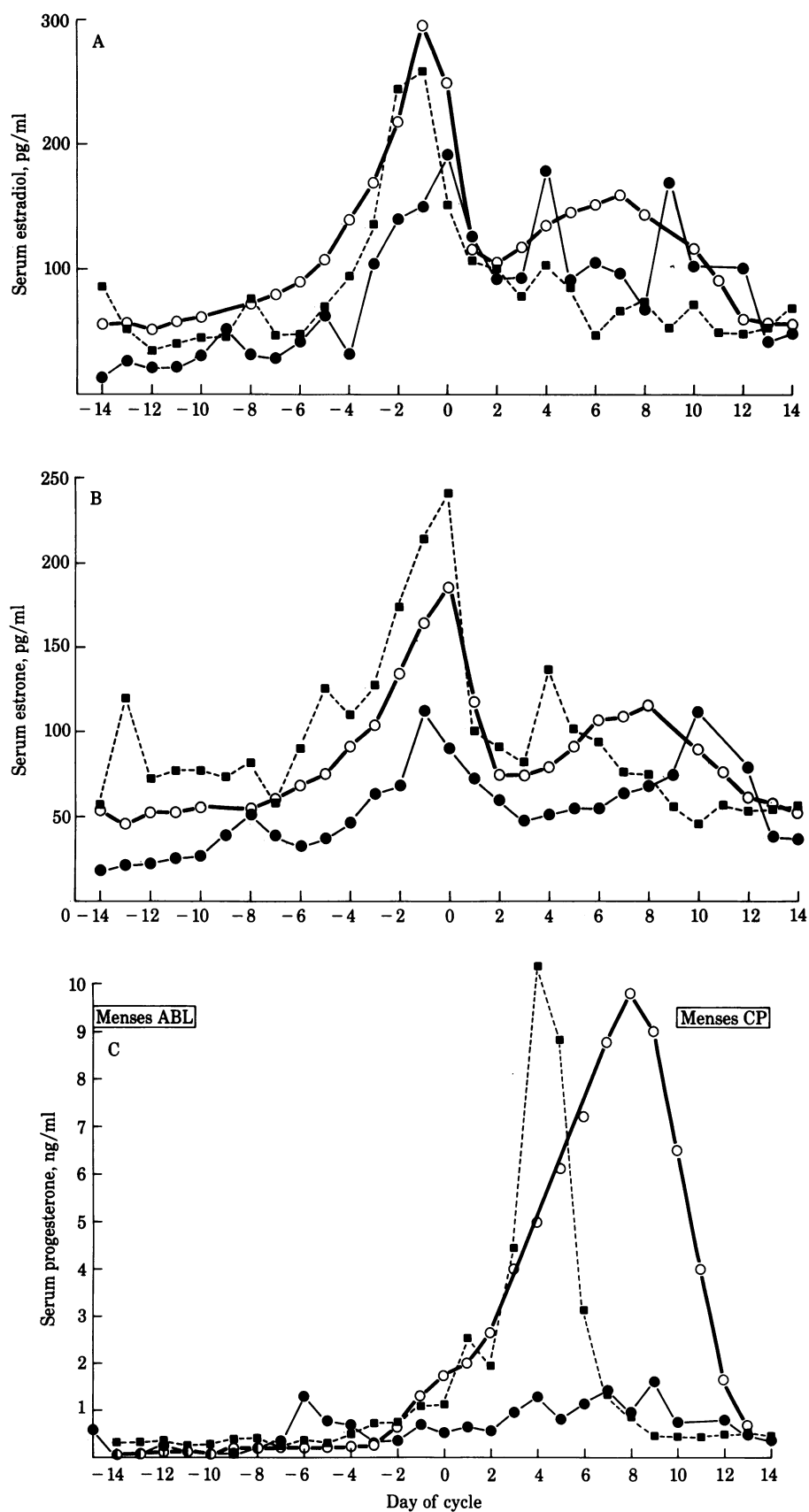
FIG. 3. Serum concentrations of estradiol (*A*), estrone (*B*), and progesterone (*C*) during the menstrual cycle in a patient with homozygous hypobetalipoproteinemia (●), in a normolipidemic control (■), and in normal women (○). Data for normal women are redrawn from refs. 25 (*A*), 26 (*B*), and 27 (*C*).

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examined. Recent studies on adrenal function in three patients with phenotypic abetalipoproteinemia (17, 18) (including the patient reported here) have shown the basal rates of corticosteroid production to be normal but, in each case, the adrenal response to prolonged (24–36 hr) stimulation with corticotropin was found to be impaired.

The present study extends these observations to an analysis of the hormone changes that occur during the menstrual cycle in abetalipoproteinemia. Our results show a marked reduction in the serum concentrations of progesterone during the luteal phase of the menstrual cycle. In normolipidemic subjects, maximal rates of progesterone secretion of 30–40 mg/day are achieved (28) in the midluteal phase of the ovarian cycle by a corpus luteum weighing approximately 1 g. Although our results provide no data on the absolute rates of progesterone secretion in this patient with homozygous hypobetalipoproteinemia, the maximum production rates of corticosteroids by her adrenals during prolonged corticotropin infusion have been found previously to be 11 to 12 mg/g of adrenal cortex (18). If it is assumed that the maximal rates of steroidogenesis in the corpus luteum and in the corticotropin-stimulated adrenal cortex of this patient are similar [studies with human corpus luteum (12) and adrenal cortical cells (2) in culture show maximal rates of secretion of progesterone or cortisol plus dehydroisoandrosterone sulfate that are similar], then the potential maximal daily production rate of progesterone in this patient would amount to only 11 to 12 mg/day.

Despite marked reductions in the luteal phase concentrations of progesterone in the patient with homozygous hypobetalipoproteinemia, serum concentrations of estradiol and estrone during the preovulatory surge fall within the normal range. This apparent discrepancy may reflect differences in the absolute rates of production of progesterone (30–40 mg/day in a normal woman) as compared with estrogens (1–3 mg/day) with greater dependency being placed on extracellular sources of cholesterol (e.g., LDL) at high rates of steroid hormone synthesis. This view is supported by studies of adrenal corticosteroid production in this patient; her basal production of corticosteroids is normal but her response to prolonged stimulation with corticotropin is clearly impaired (18).

The overall results of the present study lend strong physiological support to recent *in vitro* studies with human and bovine corpus luteum (4, 12, 13) in which plasma LDL was found to be the primary source of cholesterol for progesterone synthesis and show that, in a patient with a complete absence of plasma LDL, progesterone synthesis by her corpus luteum is impaired. Receptor-mediated uptake of plasma LDL has also been reported to serve as the principal source of cholesterol for progesterone synthesis by placental tissue maintained in culture (7). Our results suggest that the placental biosynthesis of progesterone may also be impaired in abetalipoproteinemia and that the chances for successful implantation and pregnancy may be less than normal. Although the disorder itself is rare, we are aware of only three cases of female patients with phenotypic abetalipoproteinemia who have become pregnant (29–31). In two of these cases, several miscarriages preceded an ultimately successful pregnancy. With the realization that plasma LDL serves as an important source of cholesterol for progesterone biosynthesis, attainment of successful pregnancy in patients with abetalipoproteinemia may logically require the therapeutic use of exogenous progesterone.

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