Studies were carried out with human placental lactogen (hPL) to elucidate its role in regulation of steroidogenesis (progesterone and estrogens) during early pregnancy in humans. Our in vitro studies with early pregnancy placenta under different doses of hPL demonstrated that this hormone could stimulate the synthesis of progesterone as well as estrogens (estrone and estradiol) from their respective precursors.

**Key words:** steroidogenesis human placental lactogen progesterone estrogens

**INTRODUCTION**

The placenta occupies a unique position among the endocrine glands. It is a temporary organ and during pregnancy it plays a central role in the endocrinology of pregnancy, producing peptide hormones, human chorionic gonadotropins (hCG) and hPL and steroidal hormones, estrogen and progesterone, which are essential for the maintenance of pregnancy. But knowledge regarding the mechanism of hormone regulation of placental function is not yet clearly understood.

During the last two decades, sufficient knowledge has accumulated regarding the functions of hCG, estrogen and progestins and their interdependence during pregnancy. During early pregnancy, the corpus luteum is sustained by hCG and secretes progesterone till the placenta is fully developed and takes over the functions of the corpus luteum (10).

Another protein hormone, human, placental lactogen (hPL), which is secreted and synthesized by the placenta from 35th day onward of pregnancy (10) is considered to be an important factor in maintaining pregnancy up to term. As pregnancy advances, the level of hPL goes on increasing but falls at once after delivery. Studies of Tomi et al. (5,6) on rabbits and rats showed that immunization with hPL caused interruption of placental function during the post-implantation period, and resulted in foetal death and foetal resorption. These authors suggested that circulating antibodies in treated animals might have interfered with placental steroidogenesis. On the other hand, in vivo studies indicated that steroidogenesis in human corpus luteum was not affected by large doses of hPL (13).
The present studies also indicated that passive immunization of pregnant antisera against early pregnancy placenta, which possessed antibodies against a particular antigen immunobiologically identical to hPL, resulted in foetal death and foetal retardation (8). These observations prompted us to carry out studies in depth, to explore the role of hPL in the regulation of steroidogenesis in the human placenta. As the corpus luteum maintains pregnancy by secretion of progesterone till 10 to 12 weeks of pregnancy and that stage the placenta takes over the function of corpus luteum, so it was deemed interesting to explore the role of hPL in the regulation of steroidogenesis in the human placenta in early pregnancy (i.e. 10 to 12 weeks).

MATERIAL AND METHODS

Radioactive steroids and chemicals: Pregnenolone-α-3H (specific activity 18 mCi/m mole), oestradiol-4-14C (specific activity 56 mCi/m mole) Androst-4-ene, 3,17, dio-3H (specific activity 8.3 Ci/m mole), progesterone 4-14C specific activity 50-60 mCi/mole were obtained from Radiochemical Centre, Amersham. The purity of the labeled steroids was checked by thin layer chromatography and scanning the chromatograms in a radiochromatogram scanner (Packard Model-7200).

Radioinert hormones: Progesterone, estradiol-17β, estrone, 5-pregnenolone, Androstone-3, 17 dione were obtained from Sigma Chemical Co., U.S.A. hPL was purchased from Nutritional Biochemicals Corporation, U.S.A.

Other chemicals: Laboratory chemicals and solvents were of analytical grade, obtained from B.D.H. (India). The solvents were further purified and distilled before use. Adenosine triphosphate (ATP), Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, triphosphopyridine nucleotide, (Sigma Chemical Co., U.S.A.) and Sucrose (Schwartz-Mann).

Collection and preparation of placental tissue: Early pregnancy placenta (10 to 12 weeks) were collected from women who underwent medical termination of pregnancy at All-India Institute of Medical Sciences Hospital, New Delhi. The placental tissue was stored in cold conditions soon after hysterotomy. The tissue was washed and minced repeatedly with chilled buffer to remove clots. This minced tissue was used for in vitro biosynthesis of progesterone from its precursor.

Subcellular fractionation: The minced placental tissue was homogenized with a Potter-Elvehjem homogenizer in sodium phosphate buffer (pH 7.0, 0.05 M). The homogenate was filtered through cheesecloth and fractionated in a Beckman preparative ultracentrifuge model-L, using rotor H-40. The nuclear fraction was obtained by centrifugation at 800 x g for 10 min. The supernatant from above was centrifuged at 17,500 x g for 30 min to separate the mitochondrial pellet and this supernatant was further centrifuged at 105,000 x g for 60 min and microsomal pellet was obtained.
in vitro biosynthesis of progesterone and estrogens:

To study the biosynthesis of progesterone, 500 mg of minced placental tissue was incubated in Kreb Ringer's phosphate buffer (0.15 M; pH 7.2) with pregnenolone-\(^{3}H\) (1.5 to 2.0 \(\mu Ci\)) in the incubating flask at 37°C for 2 hr with air as a gas phase in a Dubnoff metabolic shaker. Similarly for the study of biosynthesis of estrogens the microosomal fraction obtained from 2.5 to 5 g placental tissue was incubated with \(^{3}H\)-androstenedione (1-2 \(\mu Ci\)) suspended in 5 ml of sodium phosphate buffer (0.05 M; pH 7.0) containing sucrose (0.2 M) and nicotinamide (0.04 M) and TPNH generating system (10 \(\mu M\) TPN, 60 \(\mu M\) glucose-6-PO\(_4\) and 1.5 K unit glucose-6-PO\(_4\)-dehydrogenase) for 1 hr at 37°C in metabolic shaker with air as a gas phase (1). Four sets of incubations for progesterone biosynthesis and four sets of incubations for estrogens biosynthesis in duplicate were prepared, one out of which served as control. For progesterone biosynthesis to the other three sets 100 \(\mu g\), 200 \(\mu g\) and 500 \(\mu g\) of hPL was added respectively and for estrogen biosynthesis to the other three sets 100 \(\mu g\), 200 \(\mu g\) and 500 \(\mu g\) of hPL was added to the incubation flasks respectively.

Extraction of total radioactive steroids from tissue:

Four volumes of acetone were added to centrifuge tubes containing samples. The tubes were centrifuged in a refrigerated centrifuge at 2,500 rpm for 15 min. The supernatant was preserved and to the sediment, acetone was added again, stirred and centrifuged. The acetone extracts were pooled. The steroid from the sediment was again extracted with methanol and pooled with the acetone extract. The total extract was dried completely under nitrogen at 40-45°C. The dried extract was dissolved in 5 ml of alkaline water, pH 10, which facilitated the extraction of neutral steroids. For the extraction of estrogens, the dried extract was dissolved in 5 ml of acidic water (pH 45). To the aqueous extract, 15 ml of diethyl ether was added. The free steroid was extracted in ether. The ether extraction of the steroids from the aqueous phase was carried out thrice. The labelled steroids thus extracted were dried under nitrogen and the sample was then ready for further analysis by thin layer chromatography.

Chromatograph of the radioactive steroids:

Thin layer chromatography (TLC) was carried out on silica gel G and HF (0.25 mm) coated plates. The plates were activated at 110°C for 1 hr prior to use. The samples along with standard progesterone and estrogens (estrone and estradiol) were spotted on TLC plates, and ascending chromatography was performed in the following solvent systems for progesterone and estrogens.

standards were marked and the whole plate was divided into one cm² fractions and eluted with chloroform methanol (1:1), three times and dried under nitrogen.

**Radioactivity counting:**

Radioactivity was counted in a Tricarb liquid scintillation spectrometer (Packard Model, 3380), using simple scintillation fluid, containing 2, 5-diphenyloxazole (PPO) 4.00 gm and 1,4-bis-2 (4 methyl-5 phenyl-oxazolyl) benzene (POPOP) 100 mg, dissolved in 1000 ml of toluene.

**RESULTS**

**Influence of different doses of hPL on estrogens and progesterone biosynthesis:**

The in vitro conversion of androstenedione to estrone (E₁) and estradiol (E₂), expressed as dpm x 10⁶/gm tissue microsomes of placenta from early pregnancy, has been presented in Fig. 1. It was observed that hPL stimulated the conversion of the precursor to E₁, showing an increase of 12.3% over the control at the 100 µg dose level, followed by the maximum stimulation of 25.7% at the 200 µg dose. hPL also stimulated the synthesis of E₂ which followed a pattern similar to that of E₁. Furthermore, the conversion of androstenedione was markedly in favour of E₁ being 4 times greater than that of E₂. At the 500 µg dose the conversion was decreased to the same level as the 100 µg dose i.e. 12.3% (Fig. 1).

![Fig. 1: Dose response of hPL action on the synthesis of estrone (E₁) and estradiol-17 B (E₂). (Mean of 5 experiments ± SEM)](image-url)
progesterone biosynthesis: 

Estrogen (E₁) and estradiol (E₂) placenta from early pregnancy has hPL stimulated the conversion of the control at the 100 µg dose level. hPL also stimulated to that of E₁. Furthermore, the of E₁ being 4 times greater than decreased to the same level as the

Fig. 2 shows the progesterone synthesis in placental tissue expressed in dpm x 10⁻⁶/gm in the presence of different concentration of hPL. As is evident from the dose response curve, hPL at a concentration of 100, 200 and 500 µg per incubation stimulated the synthesis of progesterone by 29.6%, 34.1% and 35.1% respectively over the control.

DISCUSSION

Effect of hPL on placental steroidogenesis:

Our studies showed that hPL binding components existed in 800 x g pellet from early pregnancy placenta of humans (8). In these in vitro studies, a positive effect of hPL on the biosynthesis of progesterone and estrogens from their precursors in the human placenta from early pregnancy was demonstrated. As far as the in vitro biosynthesis of estrogens from its precursor Δ₄-Androstenedione - ³H in the early pregnancy placental microsomes is concerned, the results depicted that in the absence of exogenously added hPL the precursor was transformed into both estrone and estradiol. Conversion from androstenedione to estrone was 4 fold that of estradiol. The addition of hPL to the incubates resulted in a stimulation of biosynthesis of E₁ and E₂ at all the three dose levels i.e. 100, 200 and 500 µg, the maximum increase being at the 200 µg. dose (Fig. 1). This stimulatory effect of hPL may be due to the stimulation of syncytiotrophoblastic
cells indirectly stimulating the NADPH generating system which might, in turn, facilitate the hydroxylation of androstenedione on 19th position under the influence of hydroxylase and demelase enzyme (1,3,7,9). The stimulatory effect of gonadrophins and gonadotrophins on the aromatization of neutral steroids by human full term placenta when perfused in situ had also been shown (3,4). The NADP along with hCG when added to the incubation media containing minces or homogenates of human term placenta and 14C-testosterone and androstenedione it resulted in increased yield of aromatization (9,12). It is, therefore, possible to assume that the action of hPL in the human placenta from early pregnancy is mediated by cAMP which in turn activates the glycogen phosphorylase, the glycogen breakdown, produced via the pentose pathway, increases the NADPH levels and these factors are necessary for accelerating hydroxylation processes and aromatization.

The data of the present study indicated that hPL stimulated the conversion of pregnenolone-3H to progesterone at doses of 100 \( \mu \)g, 200 \( \mu \)g and 500 \( \mu \)g when the minces were incubated with the precursor in the presence of hPL for two hours (Fig. 1). It is conceivable that this stimulatory effect of hPL might be due to the stimulation of human trophoblast by hPL, which in turn stimulated the tropoblastic enzymes \( \Delta^1-3\beta \)-ol-dehydrogenase and \( \Delta^2 \)-isomerase which were responsible for the transformation of progesterone from \( \Delta^4 \)-pregnenolone which was available from foetal compartment (14). The increased secretion of progesterone in the perfusion medium of foeto-placental unit in vivo under the influence of hCG had also been demonstrated (2). The addition of co-factors increased the conversion of pregnenolone and progesterone from cholesterol by placental homogenates, but the addition of various amount of hCG had no effect on conversion of cholesterol to pregnenolone and progesterone (9). The stimulatory effect of hPL on the transformation of pregnenolone to progesterone demonstrated in the early pregnant placenta in this study might be due to the activation of adenyl cyclase which was bound to the inner side of cell membranes and activated the enzymes for energy production and specific hydroxylases. The role of cyclic AMP which could mimic the function of LH to stimulate progesterone biosynthesis in rabbit ovarian homogenates had been indicated (9,11).

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