FUNCTION OF THE HUMAN OVARY DURING PREGNANCY AS REVEALED BY HISTOCHEMICAL, BIOCHEMICAL AND ELECTRON MICROSCOPE TECHNIQUES

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ABSTRACT

The function of the human ovary during pregnancy has been determined by correlating the results of recent histochemical, biochemical and electron microscope studies on its various compartments such as the follicle, corpus luteum and stroma (or interstitial gland tissue), when exposed to an unusual steroidal and gonadotrophic environment. The cytological, histochemical and biochemical characteristics of the theca interna cells of developing follicles, the theca lutein and granulosa lutein cells of the corpus luteum and highly developed interstitial gland cells of thecal origin, which are hyperstimulated during the later stages of pregnancy, have been shown to constitute the sites for the biosynthesis of steroid hormones. Their function is most likely to be the secretion of steroid hormones rather than the storage of hormone precursor (cholesterol-containing lipids) as a result of high levels of human chorionic gonadotrophin (HCG); this has been strongly supported by the direct analysis of multiple steroid secretions collected from the ovarian vein during pregnancy. The subcellular basis of steroid hormone synthesis has also been briefly discussed.

In addition to the theca interna, theca lutein, granulosa lutein and interstitial gland cells (which are active steroid secretors), many cells are seen with foamy cytoplasm filled with lipids. These are the senescent cells which are refractory to HCG available in abundance during pregnancy. A suggestion has also been made that further comparative studies of morphological and biochemical responses of ovarian compartments as well as of their steroidal secretions during pregnancy are of considerable biological interest and form a most promising field of future research.
Opinions with regard to the function of the human ovary during pregnancy have been divergent. These differences of opinion started with the publication of some clinical reports (references in Pedersen & Larsen 1968; Mikhail & Allen 1967), in which abortion either followed or did not follow the removal of the ovary containing the corpus luteum, usually in the first trimester of pregnancy. The fact that human corpus luteum is not essential in late gestation, however, does not prove that progesterone is no longer produced in the lutein tissue of the ovary. Hence clinical experience gives no clue to the problem how long the human corpus luteum remains functional. Similarly morphological studies with the light microscope have also not been able to solve the problem, as a good deal of variation of opinions has been expressed with regard to the functional significance of the corpus luteum (see references in Nelson 1953; Nelson & Greene 1958; Pedersen & Larsen 1968). Although considerable attention has been paid to the structure of the corpus luteum at all stages of human pregnancy, little is known about the complex morphological and biochemical changes in the other ovarian compartments (follicle and »stroma« or interstitial gland tissue).

The past few years have witnessed a revival of interest in the functional significance of human ovarian compartments during pregnancy, when subjected to various new techniques of histochemistry, biochemistry and electron microscopy. It was considered useful to summarize and integrate the results of such techniques in order to get a deeper insight into the function of human ovarian compartments in relation to steroid hormone synthesis during pregnancy. Actually, correlations of steroidogenic activity with individual cell types or with cellular morphology, which have either been little studied or which have generally yielded unsatisfactory results, will be described and discussed here. Maqueo & Goldzieher (1966) have also stated that such correlations should prove an extremely important and most promising field for future investigations. According to them another field of interest, far less actively pursued, is that of the morphologic action of steroids and gonadotrophins on the ovary itself. In pregnancy, there is a chronic exposure of the ovary to an unusual steroidal and gonadotrophic environment. The discussion of the morphological and histochemical responses of the various ovarian compartments to this situation will, therefore, be of considerable interest.

The human ovary during the later stages of pregnancy shows developing follicles of large size, a corpus luteum and abundant interstitial gland tissue of thecal origin (Mossman et al. 1964; Deane et al. 1962; Maqueo & Goldzieher 1966; Guraya 1966a, 1968a,b; Govan 1968). These will be described here separately in relation to the biosynthesis of steroid hormones since they constitute separate gland cell species of internal secretion (Savard et al. 1965).
The cells of the growing follicle during pregnancy

The theca interna of developing follicles is well vascularized and consists of large secretory cells with abundant cytoplasm and vesicular nuclei (Guraya 1968a). Its cells, which are luteinized, develop the histochemical features of well-established, actively secreting steroid cells similar to granulosa lutein cells (Guraya 1968a,c,d). These histochemical features consist of the presence of abundant diffuse sudanophilic lipids (lipoproteins), several mitochondria and a few lipid granules consisting mainly of phospholipids; the diffuse lipoproteins of the theca interna cells are apparently derived from the abundantly developed agranular endoplasmic reticulum of the steroid gland cells (Davies & Broadus 1968; Fawcett et al. 1969; Christensen & Gillim 1969). Similar cytological and histochemical features have also been described for the vascularized theca interna cells of normal Graafian follicles during the menstrual cycle (Guraya 1968a). Neutral fats (triglycerides) and cholesterol and/or its esters demonstrable by means of histochemical techniques are not present in the theca interna cells of healthy follicles (Guraya 1968a). The cytological and histochemical features of the theca interna cells show that their function must be the secretion of steroid hormone rather than the storage of hormone precursor (cholesterol and/or its esters). This is strongly supported by the observations of Govan (1968) who stated that the theca around the developing follicles during pregnancy is chemically active, possessing enzymes necessary for steroidogenesis. Similar enzyme systems related to the biosynthesis of steroid hormones have also been described in the theca interna cells of developing antral follicles in the ovaries of non-pregnant women (Deane et al. 1962; Luh & Brandau 1964; Fienberg & Cohen 1965; König 1965, 1966; Brandau & Luh 1965) which form steroids in in vitro biochemical studies (Smith & Ryan 1961, 1962; Ryan & Petro 1966; Ryan et al. 1968). These in vitro biochemical studies are in good agreement with the cytological and histochemical data, which show that the vascularized theca interna cells form secretory gland cells and have enzymatic systems related to steroid hormone synthesis.

The granulosa cells lack the cytological and histochemical features of steroid secreting cells and are separated from the blood supply by the basement membrane of the follicle (Guraya 1968a). There is complete absence of luteinization of the granulosa cells. Presumably they are unable to undergo this transformation due to the lack of blood supply. In other words, they are not exposed to the action of HCG during pregnancy. The granulosa cells are indeed metabolically active and this activity suggests a protein rather than a lipid (or steroid) synthesis (Björkman 1962; Guraya 1968a, c,d). However, steroid metabolism in vitro by the granulosa cells has been studied in some investigations and valuable information has been obtained on steroid biosynthesis in these cells (see Ryan & Petro 1966; Ryan et al. 1968; Soma et al.
1969). These investigations, however, appear to express the biochemical potential of the granulosa cells and not necessarily their secretory activity in vivo.

If there is good evidence for believing that oestrogens are the principal products of the theca interna cells of Graafian follicles (Corner 1938; Harrison 1962) and possess the cytological and histochemical features of actively secreting steroid gland cells, then such follicles must secrete oestrogens during pregnancy. In the light of recent in vitro biochemical studies (Ryan & Petro 1966; Ryan et al. 1968; Soma et al. 1969), it can be stated that these follicles might also produce some other steroids, in addition to oestrogens.

Corpus luteum

The corpus luteum of pregnancy investigated with histochemical techniques (Guraya 1968b) and the electron microscope (Crisp & Desouky 1969) consists of two cell types, i.e., theca lutein cells and granulosa lutein cells, which also make up the corpus luteum of menstruation (Guraya 1968a). The localization and distribution of both the cell types in the corpus luteum of pregnancy are also the same as those described for the corpus luteum of non-pregnant women (Guraya 1968a). No intermediate cell types have been observed. The theca lutein cells are relatively smaller in size, and form definite, compact groups of cells which are distributed about the periphery of the corpus luteum as well as along the folds; the latter go deeper into the main mass of granulosa lutein cells which constitute the largest portion of the corpus luteum.

The histochemical features of both cell types (theca lutein cells and granulosa lutein cells) during pregnancy are very similar. The most notable feature is the presence of diffusely distributed sudanophilic lipids (lipoproteins) (Guraya 1968b) which apparently derive from the abundant agranular endoplasmic reticulum as observed with the electron microscope (Green et al. 1967a,b; Pedersen & Larsen 1968; Crisp & Desouky 1969; Adams & Hertig 1969a). Similar diffusely distributed sudanophilic lipids or lipoproteins constituting the ultrastructural agranular endoplasmic reticulum (Green & Maqueo 1965; Gillim et al. 1969; Adams & Hertig 1969b) have also been described in the luteal cells of non-pregnant women as well as of other mammalian species (Guraya 1968a,c,d, 1969a). The cytoplasmic organelles, such as the Golgi complex and mitochondria, are well differentiated even in the luteal cells studied at term; the mitochondria are large, numerous and contain tubular cristae and electron dense matrices (Green et al. 1967a,b; Pedersen & Larsen 1968; Crisp & Desouky 1969; Adams & Hertig 1969a). According to Green et al. (1967a,b), the cytoplasmic organelles of luteal cells studied at term are quite similar in appearance to those observed in the corpora lutea of the menstrual cycle (Green & Maqueo 1965; Guraya 1968a; Gillim et al. 1969:
Adams & Hertig 1969b) and pregnancy (Pedersen & Larsen 1968; Adams & Hertig 1969a). However, the most conspicuous feature of theca lutein and granulosa lutein cells at term is the absence of sudanophilic steroidal lipid droplets in the highly developed cytoplasm (Guraya 1968b). This is also in good agreement with the electron microscope observations of Green et al. (1967a,b) who stated that the lipid bodies are rarely seen in the luteal cells at term. However, some lipid granules consisting of phospholipids or phospholipids and triglycerides are present (Guraya 1968b); no Schultz-positive substances (cholesterol and/or its esters) demonstrable by histochemical techniques are present in the cytoplasm. Some lipid inclusions also occur in the granulosa lutein cells of gestation (Pedersen & Larsen 1968; Adams & Hertig 1969a). The high levels of HCG present during pregnancy (Simmer 1968) have been considered to be responsible for the complete absence of accumulations of sudanophilic lipids in the cells of the corpus luteum (Guraya 1968b) since the gonadotrophins are now well known to cause the depletion of cholesterol-containing lipids from the ovarian gland cells of internal secretion (Guraya & Greenwald 1964a,b, 1965; Guraya 1967a,b, 1968e). Active steroidogenesis by means of stimulation by gonadotrophin is considered to occur only when there is no storage of hormone precursors (cholesterol and/or its esters) in the cytoplasm of steroid gland cells (Guraya 1967a). Corresponding to the rising levels of progesterone and oestradiol during the luteal phase of the menstrual cycle, cholesterol and/or its esters, demonstrable by histochemical techniques, are not seen in the luteal cells, which, however, show some lipid granules consisting of either phospholipids or phospholipids and some triglycerides (Green & Maqueo 1965; Guraya 1968a). However, the 9- to 10-day old luteal cells in the human ovary begin to store hormone precursor (steroidal lipids) (Guraya 1968a). This is clearly related to the fall in steroid hormones production, which begins on day 9 after ovulation. Thus, it seems reasonable, to assume that the theca lutein and granulosa lutein cells which show no steroidal lipids during the later stages of pregnancy, must be involved in the secretion of steroid hormones rather than in the storage of hormone precursors as a result of the marked HCG stimulation (Guraya 1968b, 1969b). Green et al. (1967a,b), after studying these cells with the electron microscope, have also arrived at similar conclusions with regard to the functional significance of corpus luteum cells at term. Enzymatic activities indicative of biosynthesis of steroid hormones have been demonstrated histochemically in the corpus luteum of pregnancy (Deane et al. 1962); however, they disappear from the regressing luteal cells. Thus, both the ultrastructural and histochemical features of theca lutein and granulosa lutein cells during pregnancy, as discussed above, show that they are engaged in active steroid production. This view is also supported strongly by biochemical studies which have shown that the corpus luteum at term shows the highest levels of enzymes involved in the production of TPNH
Evidence for the increased production of TPNH in tissue is suggestive of an active biosynthesis of steroid hormone, since it is a cofactor in the gonadal biosynthesis of steroid hormones (see Nielson & Warren 1965). The in vitro biochemical studies have shown that the corpus luteum of pregnancy can synthesize progesterone (Hammerstein et al. 1964; Rice et al. 1964; Savard et al. 1965; Ryan 1963). LeMaire et al. (1968), using in vitro biochemical techniques, have also demonstrated that the term corpora lutea produced a substantial conversion of acetate to progesterone. Davis et al. (1965) and Davis & Plotz (1957) concluded from investigations with labelled acetate and cholesterol that the corpus luteum produces cholesterol up to the end of pregnancy. Hence they also considered the possibility that progesterone is produced up to the end of pregnancy. Zander (1959) and Zander et al. (1958) have isolated progesterone from corpora lutea from the second to the tenth month of pregnancy; corpora lutea of the tenth month of pregnancy still contained progesterone in quantities from 3.6 to 15.0 mg per gram of tissue. In line with observations of Zander (1959) and Zander et al. (1958) who suggest that the corpus luteum continues to synthesize progesterone throughout pregnancy, Δ5-3β-hydroxysteroid dehydrogenase activity also persists in the corpora lutea even at term (Deane et al. 1962). All the ultrastructural, histochemical and biochemical studies on the corpus luteum of pregnancy as discussed above are in good agreement with the observations of Mikhail & Allen (1967) and LeMaire et al. (1970) who have demonstrated the presence of significant amounts of progesterone in the ovarian venous blood, indicating that the corpus luteum of pregnancy is a functioning endocrine organ throughout gestation. However, following delivery, the corpus luteum of pregnancy undergoes an abrupt loss of oxidative enzymic activity, indicating complete loss of secretory potential (Deane et al. 1962).

**Interstitial gland tissue**

The ovaries of pregnant women during the later stages of gestation show marked hypertrophy and development of interstitial gland cells (Mossman et al. 1964; Guraya 1966a) which are clearly formed by a «luteal-like» transformation of theca interna and surrounding fibroblastic stromal tissue of degenerating follicles. The interstitial gland cells of pregnancy form relatively thicker and more compact zones around the remnants of follicular cavities. They are more highly vascularized than those in the non-pregnant state (Guraya 1967c). The individual gland cells are of larger size, and show a considerable cytological differentiation than those of non-pregnancy as evidenced by the greater development of cell organelles such as the Golgi zone and mitochondria (Guraya 1966a). Such cell organelles are relatively less developed in the corresponding cells of non-pregnancy (Guraya 1967c). The
cells of both non-pregnancy and late gestation resemble each other with regard to the presence of diffuse sudanophilic lipoproteins, which have been assumed to constitute the highly developed agranular endoplasmic reticulum of ultrastructural studies (Muta 1958; Davies & Broadus 1968; Beltermann & Stegner 1968; Christensen & Gillim 1969). The most prominent feature of interstitial gland cells of late pregnancy is the lack of sudanophilic steroidal lipids (Guraya 1966a) which accumulate in the cells of non-pregnancy (Guraya 1967c) as well as in the ovarian interstitial gland cells of other mammalian species in certain physiological situations (Guraya & Greenwald 1964a,b, 1965; Guraya 1966b, 1967a,b, 1968f,g). However, the relatively mature cells studied at term show many vacuoles (Guraya 1966a) which first originate in the Golgi zone; the vacuoles, which react negatively with the histochemical tests used, may represent the transitory reservoirs of some steroid hormones; the latter are immediately extruded out as judged from the presence of vacuoles near the plasma membrane. The lack of steroidal lipids in the interstitial gland cells of late pregnancy has also been attributed to marked HCG stimulation (Guraya 1966a). From both the cytological and histochemical features it has been suggested by the present author (Guraya 1966a, 1967c, 1969b) that the interstitial gland cells of late gestation must be relatively more actively involved in the biosynthesis of steroid hormones than those of non-pregnancy. In other words, the cells of non-pregnancy appear to be relatively quiescent or dormant as shown by the accumulation of sudanophilic steroidal lipids (rich in cholesterol and/or its esters). The interstitial gland cell of both non-pregnancy and pregnancy are typical steroid-producing cells as far as their morphology and histochemistry are concerned. They are, however, without much qualitative differences in their organization and interrelationships. The changes can thus be assumed to be quantitative rather than qualitative.

Active steroidogenesis through stimulation by gonadotrophin is considered to occur only when there is no storage of hormone precursors (steroidal lipid droplets) in the cytoplasm of steroid gland cells. Thus it seems reasonable to assume that the function of hyperstimulated interstitial gland tissue during late pregnancy must also be the secretion rather than the storage of hormone precursors (steroidal lipids) as a result of marked HCG stimulation (Guraya 1966a, 1967c, 1968b, 1969b). This suggestion is strongly supported by the biochemical evidence of Nielson & Warren (1965) who found that the luteinized stromal tissue at term, which is probably composed of the interstitial gland cells under discussion, showed the highest activity of the pentose shunt dehydrogenases. According to Rice & Savard (1966), the highest incorporation of [1-14C] acetate into androgenic steroids was also found in the ovarian stroma obtained during pregnancy, which can also be presumed to contain the interstitial gland cells under discussion. If the formation of androgenic steroids as the major product of the ovarian stroma, as suggested by Savard
et al. (1965), Rice & Savard (1966) and Leymarie & Savard (1968), is true, then the highly developed interstitial gland tissue of late pregnancy, which has been shown to be very active in the biosynthesis of steroids, may be the source of plasma testosterone in women both in late pregnancy and immediately after delivery (Demisch et al. 1968). This may be interpreted as reflecting the effect of endogenous HCG on testosterone formation in the ovarian stroma (interstitial gland tissue).

As well as the hypertrophied interstitial, theca lutein and granulosa lutein cells (which are active steroid secretors) described above, the human ovary during late gestation also shows many hypertrophied cells with foamy cytoplasm, which are distributed either in the stroma or at the periphery of some clear scars (Maqueo & Goldzieher 1966; Guraya 1969b). Their highly developed cytoplasm is filled with deeply sudanophilic lipids consisting of pigments, triglycerides, cholesterol and/or its esters and some phospholipids (Guraya 1969b). The accumulation of these lipids suggests that the cells with foamy cytoplasm are refractory to gonadotrophic substance (HCG) available in abundance during pregnancy, which is believed to be responsible for the lack of cholesterol-containing lipids in other gland cell species (theca interna, theca lutein, granulosa lutein and interstitial gland cells) as already discussed. These senescent cells are seen to be derived from the residual cells of interstitial gland tissue and theca lutein tissue in the ovaries of non-pregnant women (Guraya 1969b).

**DISCUSSION AND CONCLUSIONS**

The electron microscope, histochemical and biochemical studies including in vitro experiments as integrated here, reveal that the function of different steroid gland cell species during pregnancy must be the secretion of steroid hormones rather than the storage as a result of high levels of HCG which is known to stimulate steroidogenesis in vivo and in vitro (Savard et al. 1965; Eik-Nes 1964; Guraya 1967a). This conclusion is strongly supported by the studies of Mikhail & Allen (1967) who have collected multiple steroid secretions such as progesterone, 17α-hydroxyprogesterone, 20α-hydroxy-pregn-4-en-3-one and androstenedione from the ovarian vein. Since progesterone, 17α-hydroxyprogesterone and 20α-hydroxy-pregn-4-en-3-one are the major steroids formed from radioactive acetate by the human corpus luteum of both menstruation and pregnancy (Savard et al. 1965; LeMaire et al. 1968), the presence of significant amounts of progestins in the ovarian venous blood, combined with the cytological, histochemical and biochemical features of theca lutein and granulosa lutein cells indicate that the corpus luteum of pregnancy is a functional endocrine organ throughout gestation.
The contribution by other compartments of the ovary (e.g., follicle, interstitial gland tissue) to the total steroidal secretion must also be evaluated during pregnancy. However, androstenedione isolated from the ovarian vein by Mikhail & Allen (1967) can be presumed to be the product of hyperstimulated interstitial gland cells since androgens (e.g., androstenedione, dehydroepiandrosterone and testosterone) are the major secretory products of human ovarian interstitial gland tissue (or stroma) as found in studies with in vitro experiments. According to Savard et al. (1965), Rice & Savard (1966) and Leymarie & Savard (1968) the synthesis of radioactive androst-4-ene-3,20-dione seems to be the most sensitive index of response to the effect of HCG and LH in vitro. Direct isolation of an androgen, androst-4-ene-3,20-dione, from pooled medullae of human ovaries by Simmer & Voss (1960) also suggests that this ovarian compartment might be the site of androgen formation. The present author believes that the biosynthesis of steroid hormones by the ovarian stroma is actually due to the presence of hypertrophied interstitial gland cells since the compressed, spindle-shaped stromal elements proper constituting the general ovarian stroma, do not possess the cytological, histochemical and biochemical features of well established steroid-secreting cells.

Since 17β-oestradiol and oestrone are considered to be the major radioactive products formed from [1-14C] acetate by the human antral follicle, such steroids can be assumed to be also secreted in vivo, as the ovary during pregnancy contains developing antral follicles with the glandular appearance of theca interna cells, which possess the features of actively secreting steroid gland cells.

Actually the importance of steroidal secretions by the various compartments of hyperstimulated ovary during later stages of gestation has been overlooked because of the fact that the placenta alone is sufficiently adequate to supply the needs of gestation. Since the human ovary during pregnancy is exposed to an unusual steroidal and gonadotrophic environment, further comparative studies of morphological and biochemical responses of different ovarian compartments as well as of their steroidal secretions during these various situations are of considerable biological interest and should form most promising fields for future research. Steroid analyses of effluent ovarian blood give no indication of the ovarian elements responsible for the actual synthesis of a particular steroid. Our approach, therefore, should be to study the theca interna, granulosa lutein and interstitial gland cells separately, all of which possess similar cytological, histochemical and biochemical features related to biosynthesis of steroid hormones. The author has suggested that the appearance of diffuse lipoproteins in steroid gland cells can serve as a useful histochemical indicator of their luteinization and function (see Guraya 1969c; Upadhyay & Guraya 1971). The presence of abundant diffuse lipoproteins in the various steroid gland cell components of the human ovary during later stages of
gestation, correlated with other parameters described here, can also be considered a good indicator of their function. Correlated morphological and biochemical studies of other investigators have shown that the membranes of smooth reticulum (or diffuse lipoproteins) play an important role as sites for enzymes involved in steroidogenesis (Christensen 1965; Goodman et al. 1968; Fawcett et al. 1969; Christensen & Gillim 1969). In addition to acting as a site for the synthetic enzymes involved in the biosynthesis of steroids, smooth reticulum or diffuse lipoproteins may also accumulate and store cholesterol as a constituent of their lipid component. In this way precursor cholesterol would be stored in close spatial relation to the enzymes associated with diffuse lipoproteins (or membranes of smooth reticulum); these enzymes catalyze the conversion of cholesterol to biologically active steroid hormones. The extent of the diffuse lipoproteins in the different steroid gland cell components of the human ovary during late pregnancy might therefore, be in part, an expression of the amount of cholesterol accumulated. From this discussion it can be concluded that the diffuse lipoproteins of different steroid gland cell components of the human ovary during late pregnancy play an important role in steroidogenesis. The increase in the size of mitochondria as well as complex alterations in their inner profiles concomitant with the development of diffuse lipoproteins (or membranes of smooth reticulum) may also result in their participation in steroid metabolism (Fawcett et al. 1969; Christensen & Gillim 1969).

Note added in proofs: The conclusion that the human corpus luteum is a functioning endocrine organ throughout gestation as a result of high levels of HCG is further supported by the recent studies of Le Maire et al. (Amer. J. Obstet. Gynec. 110 (1971) 612).

ACKNOWLEDGMENTS

Some of the author’s investigations on the human ovary cited in this paper were carried out in the Human Reproduction Laboratory, Department of Obstetrics and Gynecology, University of Kansas Medical Center, Kansas City, Kansas during the tenure of postdoctoral fellowship of the Population Council, New York.

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Received on March 3rd, 1971.