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# PLACENTAL STEROID HORMONES

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SUMMARY

Little if any *de novo* synthesis of cholesterol occurs in mid-gestation placenta. The utilized cholesterol comes from foetal and maternal sources. The main products from cholesterol metabolism in placenta are pregnenolone and progesterone. The placental metabolism of progesterone is limited. Minute amounts of 6β-hydroxyprogesterone and 20α-dihydroprogesterone are formed. One of the most important reactions in the placenta is the aromatization of $C_{19}$-precursors. In this connection the sulfatase, the 3β-hydroxysteroid dehydrogenase, the 19-hydroxylase and the 17β-hydroxysteroid dehydrogenase are of special interest, also the possible regulation or inhibition of these enzymes. The reducing capacity of the placenta is limited. In comparison to human placenta great species differences in biotransformation of steroids may occur. The inhibition of estrogen biosynthesis by steroids will be considered.

## INTRODUCTION

Pregnancy in humans is characterized by a gradual increase in urinary excretion of gestagenic and oestrogenic steroids. The 10–1000 fold increase of these steroids has been assumed to reflect the steroidogenetic function of the placenta. Yet in 1964 Diczfalusy and his coworkers showed that the changed steroid metabolism represents the capacity of placenta and foetus together [1, 2].* In the time up to 1973 several reviews in this field were published [3–6]. Therefore, in the following only the mainlines of this earlier work will be treated.

## 1. BIOGENESIS

Both the placenta and the foetus are incomplete systems for biosynthesis and metabolism of steroid hormones, because in both certain enzymes are lacking. But foetus and placenta are a unit, which complete one another in their enzyme systems. For example: little, if any *de novo* synthesis of cholesterol occurs in mid-gestation placenta, but an abundant *de novo* synthesis takes place in the human foetus. Yet the degradation of the cholesterol side-chain takes place mainly in the placenta. The compartments foetus and placenta form, with the mother, a functional endocrine unit.

### 1.1 *Progesterone*

As already mentioned cholesterol *de novo* synthesis from acetate plays only a minor role in placenta. The utilized cholesterol comes from foetal and maternal sources [7]. The next metabolic step, side-chain cleavage, takes place mainly in the placenta. The main products from cholesterol metabolism in placenta are pregnenolone and progesterone. The foetus converts cholesterol to pregnenolone, pregnenolone sulfate and dehydroepiandrosterone sulfate. Foetal steroid synthesis seems to be characterized by the predominance of sulfate conjugated pathways, while in placenta, in general steroid sulfates are hydrolysed before being metabolized. Indeed, there has been described the direct side-chain cleavage of cholesterol sulfate to form pregnenolone sulfate [8], but this reaction does not play an important role.

It is well established that the main part of progesterone is formed in the placenta. This was demonstrated in cases with foetal death or abdominal pregnancy or in experiments with ligation of the umbilical cord [9]. At term the placenta produces about 250 mg progesterone per day, of which 75 mg are delivered to the foetus [6, 10, 11].

### 1.2 *Oestrone–oestradiol*

The placenta is lacking the enzyme combination 17α-hydroxylase and desmolase for the removal of the pregnane side-chain. So for androgen and oestrogen biosynthesis the placenta has to pass pregnenolone or progesterone to the foetus for splitting off the side-chain.

One of the most important reactions in the placenta is the biosynthesis of oestrogens by aromatization of $C_{19}$ precursors. The main precursor is dehydroepiandrosterone which appears as sulfate mainly from the foetal and maternal adrenal. Substantial quantities are present in cord blood [12, 13]. The foetus has a high sulfuryltransferase activity in the liver. It is suggested that this conjugation with sulfuric acid may be a protection against biologically active steroids. In contrast to the foetus the placenta is lacking this enzyme.

* Many groups all over the world were engaged in these investigations.

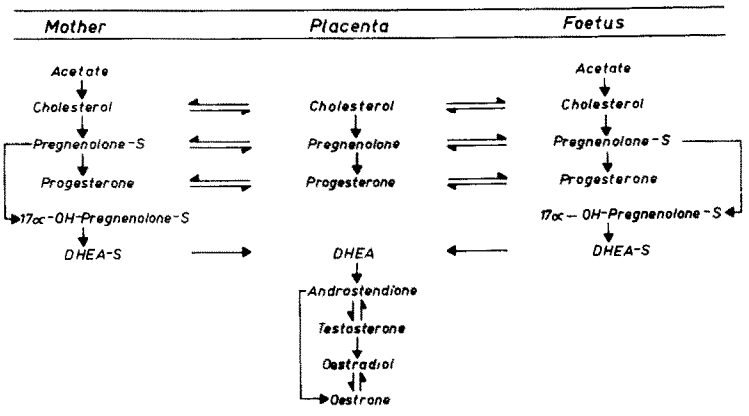


Fig. 1. Mainlines of the biogenesis of placental steroid hormones.

The placenta contains a sulfatase to hydrolyze sulfates. Because favourably unconjugated steroids are utilized for steroidogenesis by the placenta the sulfatase possibly plays a major role in regulating oestrogen biosynthesis.

Evidence was obtained that the aromatization takes place most probably *via* α,β-unsaturated 3-ketosteroids as androstenedione and testosterone [14, 15]. Therefore the reaction dehydroepiandrosterone to androstenedione, catalyzed by the enzymes 3β-hydroxysteroid dehydrogenase and 5-en-4-isomerase are of importance. The next step necessary for aromatization is 19-hydroxylation of testosterone and androstenedione. Although the 19-hydroxylation of dehydroepiandrosterone was demonstrated as well as the aromatization of 19-hydroxydehydroepiandrosterone [56] other results indicated, that, after inhibition of the 3β-hydroxysteroid dehydrogenase 19-hydroxylation of dehydroepiandrosterone is not a significant alternative pathway to oestrogens in the human placenta [52]. Products of aromatization in the placenta are only oestrone and oestradiol. No oestriol is formed without the foetus being involved [16]. In *in situ* placenta perfusion studies the relationship of synthesized oestrone to oestradiol was 1:3 in placental tissue, but 3:1 in the perfusate irrespective of the precursor used [14, 17].

The enzymes 3β-hydroxysteroid dehydrogenase, the 5-en-4-isomerase and the 19-hydroxylase are all present in the placenta.

The aromatization altogether is stimulated by HCG and by prostaglandins [18, 19].

## 2. METABOLISM

Large amounts of metabolites are formed in the foetoplacental–maternal compartments. There are marked differences where special metabolic reactions occur. Our knowledge about possible activities or the functional role of metabolites in general is limited, but we are now in a phase, where metabolites are involved in the interpretation of the mechanism of action or are connected with the finding of special activities under experimental conditions. For example, a 5α-dihydroderivative of progesterone [20] and 2-hydroxyoestrogens [21] have become interesting of late. Metabolites of this type may have antihormonal activities on the basis of receptor bindings. Another aspect is the possible inhibition of steroid transforming enzymes by some metabolites.

### 2.1 *Gestagens*

The reducing capacity of the placenta is only limited. The quantitatively most important reaction is the reduction of the 20-keto group in progesterone to 20α- and 20β-hydroxyderivatives. In human placenta 20α-dihydroprogesterone is the final main metabolite of progesterone. The same metabolite is formed in the foetus too.

Pregnenolone and pregnanediol which are excreted in human pregnancy in large amounts, are mostly formed in the maternal circulation.

Ring A reduction of progesterone is only of limited importance. As mentioned, in human placenta 20α-dihydroprogesterone is the end product. But in other species reduction to 5α,3-keto, 5α,3β-hydroxy- and 5α,3α-hydroxy-derivatives were found. These species differences will be treated later. Also the 4-ene-3-keto-group in $C_{19}$-steroids is reduced in placenta. 5α and 5β derivatives are formed depending on the species. Not so much hydroxylation reactions were found for progesterone. Pregnenolone was metabolized by human, rhesus monkey, baboon and chimpanzee placenta to 6β-hydroxyprogesterone [22, 23] and with human placenta to 6α-hydroxyprogesterone [23]. This was the first demonstration of a 6α-hydroxylation of $C_{21}$-steroids by human tissue. Yet, this 6-hydroxylation does not play an important role in progesterone metabolism.

### 2.2 *Oestrogens*

In $C_{18}$- and $C_{19}$-steroids reduction of the 17-keto-group is performed by placenta. For human placenta, the reduction of the 16-keto-group to 16α- and 16β-hydroxy-derivatives was described [24].

Several hydroxylation reactions were observed. Hydroxylations of $C_{19}$-steroids are to be included,

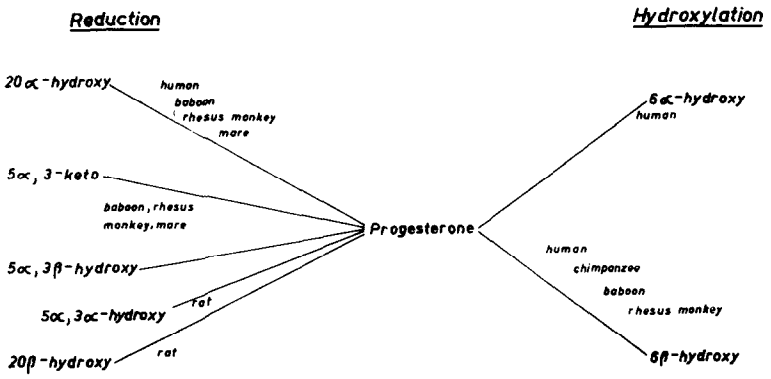


Fig. 2. Metabolism of progesterone in placenta.

because in this way precursors of substituted oestrogens are received.

The most important reaction is the 19-hydroxylation of $C_{19}$-steroids. 19-Hydroxydehydroepiandrosterone, 19-hydroxytestosterone and 19-hydroxyandrostenedione are intermediates in oestrogen biosynthesis.

Beside 19-hydroxylation the most important hydroxylation in humans is in 16-position to come to oestriol. 16-Hydroxylation of $C_{19}$-precursors or oestrogens does not take place in the placenta. The placenta contains no 16-hydroxylase. This enzyme is exclusively located in the foetus. 16α-Hydroxydehydroepiandrosterone is the quantitatively most important precursor for oestriol in pregnant women, the use of 16α-hydroxytestosterone is more limited [25].

It is generally accepted that oestriol assays give a useful indication of foetal well being because there is a foetal and a placental part in the production of this steroid.

An increase of 2-hydroxylated oestrogens in pregnancy has been reported [26]. 2-Hydroxyoestrone was not only received from aromatization of 2β-hydroxytestosterone, but also from direct hydroxylation of oestradiol [27]. It is proposed that the alternative metabolic pathways of oestradiol (16α-hydroxy or 2-hydroxy) and the direction of oestradiol metabolism may have an important role in the modulation of oestrogenic activity [21].

The oestrogen precursor dehydroepiandrosterone was hydroxylated by human placenta in 7α- and 7β-position [28, 29].

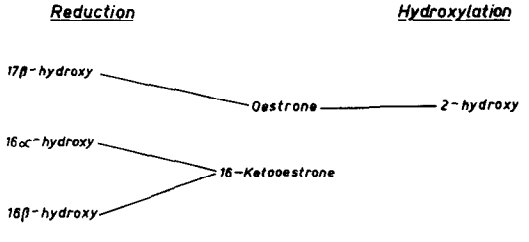


Fig. 3. Metabolism of oestrogens in placenta.

## 3. SPECIES DIFFERENCES

Most investigations were done with human placenta, not so much is known about other species. The following species differences in steroid biosynthesis and metabolism are found.

*In situ* perfusion of rhesus monkey placenta with dehydroepiandrosterone showed, that aromatization is very limited [30]. These data would explain the low oestrogen excretion in pregnant rhesus monkeys compared to humans. An aromatization of testosterone [31] respectively of androstenedione [32] with rat placenta and the aromatization of testosterone with mouse placenta [33] could not be demonstrated. Baboon placenta hydroxylated testosterone in 2β-position [34]. Pregnenolone was metabolized by human, rhesus monkey, baboon and chimpanzee placenta to 6β-hydroxyprogesterone [22, 23] and only by human placenta to 6α-hydroxyprogesterone [23].

Oestriol is a typical human excretion product in pregnancy. Among apes, only the gorilla seems to have a substantial ability to 16α-hydroxylate [35].

With human placenta and the placenta of baboons, rhesus monkeys and the mare, reduction of progesterone leads to 20α-hydroxy-derivatives [36–39], while rat placenta reduces to 20β-hydroxy compounds [32]. In squirrel monkey [38] and rabbit placenta [40] no 20-keto-group reduction took place.

In human placenta 20α-dihydroprogesterone is the end product, but in placentas of baboons, rhesus monkeys and mares reduction of 4-ene-3-keto-group to 5α,3-keto- and 5α3β-hydroxy compounds takes place [38, 39] while in rat placenta 5α, 3α-hydroxy-derivatives were formed [32, 41]. Another role may play the reduction of the 4-ene-3-keto-group in $C_{19}$-steroids. In human placenta 5α- and 5β,3-keto compounds are received as well as 5α,3β-hydroxy-derivatives [42, 43]. In mice [33] and rat [32] placenta reduction to 5α,3α-hydroxy and 5α,3β-hydroxy-derivatives took place. In rats an extensive reduction of ring A was observed, while, as already mentioned, no aromatization of $C_{19}$-steroids is observed. Since 5α,3-keto-androstanes are not aromatized by placenta, the formation of ring A reduced products rep-

Table 1. Inhibition of steroid-3-sulfatase

| | | |
|---|---|---|
| pregnenolone | dehydroepiandrosterone | oestrone |
| 17-OH-pregnenolone | 16α-OH-dehydroepiandrosterone | oestrone sulfate |
| 20α-dihydropregnenolone | androstenedione | oestradiol |
| 20β-dihydropregnenolone | testosterone | 2-OH-oestradiol |
| pregnenolone sulfate | 16α-OH-androstenedione | oestriol |
| progesterone | 19-OH-androstenedione | 2-OH-oestriol |
| 17-OH-progesterone | 16α-OH-testosterone | |
| 20α-dihydroprogesterone | androsterone sulfate | |
| 20β-dihydroprogesterone | etiocholanolone | |

resents a potentially competitive reaction to oestrogen biosynthesis.

#### 4. INHIBITION OF KEY ENZYMES IN BIOSYNTHESIS

In the last few years the effect of steroid hormones or their metabolites on steroid transforming enzymes has become more and more interesting. A sort of self regulation of biosynthesis and metabolism is possible in this way. Three enzymes of placenta will be treated here.

### 4.1 *Sulfatase*

In placenta in general steroid sulfates are hydrolyzed before being metabolized. A sulfatase capable of hydrolyzing steroid-3-sulfates is present in placenta and it was demonstrated, that placenta is the richest human source of this activity [44]. The steroid-3-sulfatase is inhibited by several endogenous steroids some of them are summarized in Table 1.

Inhibiting activity have pregnenolone, pregnenolone sulfate, oestrogens, oestrone sulfate, $C_{19}$-intermediates, $C_{19}$-metabolites and excess substrate [45, 46]. The inhibitory effects are cumulative and therefore the inhibition of the sulfatase is discussed as a mechanism for control of placental oestrogen biosynthesis.

### 4.2 *3β-Hydroxysteroid dehydrogenase*

Another key enzyme is the 3β-hydroxysteroid dehydrogenase, an enzyme, which is essential for the biosynthesis of progesterone and oestrogens in pregnancy. In human foetus this enzyme is not present. Only the placenta is responsible for the reaction pregnenolone to progesterone and dehydroepiandrosterone to androstenedione [47]. The 3β-hydroxysteroid dehydrogenase may be a rate limiting enzyme, its activity is inhibited by several steroids known to be present in the placenta. So this inhibition may be part of a feedback system to control placenta steroid biosynthesis.

A lot of steroids were tested for inhibitory activity. Some are summarized in Table 2.

With progesterone and its two 20-dihydroderivatives an appreciable inhibition of the reaction pregnenolone to progesterone could be demonstrated [48]. Progesterone is effective in concentrations similar to that found in placenta. Other strong inhibitors are oestradiol and androstenedione [49], both substances are also present in the placenta. It is remarkable that the reaction dehydroepiandrosterone to androstenedione is neither inhibited by progesterone nor by oestradiol. Only androstenedione exerts a fairly strong activity. Two different enzymes are therefore discussed as present in the placenta [49].

### 4.3 *Aromatizing enzyme system*

In the last few years several experiments were done concerning inhibition of placental aromatization. As far as it concerns inhibition by endogenous steroids, it will be possible to get more insight in regulation of hormone production and mechanism of biosynthesis. Inhibition of aromatization by steroidal drugs may be useful in oestrogen excess disorders, as was shown for males with gynecomastia [51]. By aroma-

Table 2. Inhibition of 3β-hydroxysteroid dehydrogenase

| steroid | pregn. → prog. | dehydroepi-androsterone → androstene-dione | ref. |
|---|---|---|---|
| oestradiol | + + + | | 49 |
| | | — | 50 |
| oestrone | + + | — | 49,50 |
| androstenedione | + + + | + + | 49 |
| testosterone | + | | 50 |
| dehydroepi-androsterone | + + | | 50 |
| progesterone | + + + | — | 48,49 |
| 20α- and 20β-dihydroprogesterone | + + + | | 48 |
| 17α-hydroxy-pregnenolone | — | | 50 |

Table 3. Inhibition of aromatizing enzyme system

| steroid | inhibition | ref. |
|---|---|---|
| oestradiol | − | 52 |
| oestrone | − | 52 |
| oestriol | + | 52 |
| 2-hydroxyoestrone | − | 53 |
| 6α-hydroxyoestrone | − | 53 |
| 15α-hydroxyoestradiol | − | 53 |
| progesterone | + | 52,53 |
| 20α-dihydroprogesterone | − | 53 |
| 5α-androstane-3,17-dione | +++ | 52 |
| 5α-dihydrotestosterone | +++ | 52,53,54 |
| androst-4-en-19-ol-3,17-dione | +++ | 52 |
| 6β-hydroxytestosterone | +++ | 53 |
| 6β-hydroxyandrostenedione | +++ | 52 |
| androst-4-ene-3,6,17-trione | 6+ | 52 |

tase inhibition physiological control of oestrogen synthesis may be achieved. This may be also the case in steroid induced regression of some breast cancers.

In Table 3 some steroids are summarized, which inhibit aromatization and are sometimes present or possibly present in placenta.

End product inhibition was not found. All oestrogens, including hydroxylated metabolites, are ineffective or weakly effective. Progesterone is only weakly effective, the 20α-dihydro-derivative is ineffective. From the $C_{19}$-steroids only 5α-reduced metabolites and 19-hydroxylated intermediates are effective. Interesting, but not of physiological importance, is the strong effect of 6-oxygenated $C_{19}$-steroids.

## 5. FACTORS INFLUENCING STEROID BIOSYNTHESIS

### 5.1 *Choriongonadotropin and placental lactogen*

The proteohormones are synthesized entirely in the trophoblast and secreted directly into the maternal circulation. Only small amounts are found in the foetus. Therefore the concept of foeto-placental unit is not applied to them.

In perfusion studies it was shown that the aromatization of $C_{19}$ precursors was increased by HCG [57, 58]. Also LH increases the placental aromatization [59]. There is some evidence that the increase in aromatization was restricted to the hydroxylation of $C_{19}$ and that HCG was not effective on any of the steps after the initial hydroxylation [60]. Also a stimulation of aromatization in human placenta by human placental lactogen was observed [61].

### 5.2 *Prostaglandins*

The oestrone and oestradiol production of the intact human placenta *in vitro* was enhanced to a high degree by perfusion with prostaglandin $E_1$, and to a lesser degree by prostaglandin $F_{2\alpha}$ or prostaglandin $E_2$ [18, 19]. Prostaglandins may increase oestrogen production by stimulating adenylcyclase.

### *Concluding remarks*

Only some main points are summarized in this short review. Not all aspects could be treated in this paper. In the last decade it has been accepted that there are special enzyme deficiencies in the placenta and the foetus for steroid biosynthesis and metabolism. The concept of the foeto-placental unit has helped our understanding of the hormonal function of the placenta. Many questions arise from the marked species differences in biosynthesis and metabolism. Our knowledge about the functional role of metabolites is limited. The possible regulating or modulating action of steroids may be a fruitful field for further investigations.

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## DISCUSSIONS

*Arai.* Thank you very much for your beautiful presentation. I wonder if you have calculated the inhibition constant of estriol for instance in the case of 3$\beta$-hydroxysteroid dehydrogenase and also of the aromatizing enzyme system.

*Schubert.* The inhibition constant ($K_i$) of estriol in the case of 3$\beta$-hydroxysteroid-NAD-oxidoreductase from Pseudomonas testosteroni is 1.6 $\mu$M (strong inhibition). In the case of the placental aromatizing enzyme system the inhibition was percentagely related to the transformation of androstenedione to estrone without inhibitor. Estriol inhibited about 16%.

*McNaughton.* I was most interested in your work on the inhibition effect of some of the steroids. I think this might have some practical importance because a number of compounds are used in early human pregnancy, for example, 17-hydroxyprogesterone and some of its esters have been used and I wondered if you had either done any work or could speculate as to whether these compounds which are used therapeutically might in fact have inhibitory effects on the production of steroids by the placenta. I think this might be of some practical importance.

*Schubert.* It may be possible that certain enzyme inhibiting effects play a role in the therapy with gestagens. But up till now clear cut relations are not to be seen.

*Denton.* I was very interested in your attention to the 17,20$\alpha$, and 17,20$\beta$-dihydroxy-4-pregnen-3-one and also pregnenolones. I am wondering if you could tell me something of their rate of secretion in normal pregnancy and under conditions such as eclampsia and pre-clampsia. My reason for asking the question is that we have described a condition of experimental induction of hypertension by ACTH in the sheep. It turns out that the 17,20$\alpha$ and possibly the 17,20$\beta$-dihydroxy-4-pregnen-3-one are causal elements albeit under conditions of raised levels of other steroids such as cortisol. On present evidence their hypertensive action is apparently not by a mineralocorticoid or a glucocorticoid effect. So I am wondering what you could tell us about the secretion rates of these in primates.

*Schubert.* Secretion rates of the 20-dihydro compounds in primates are not known to me.

*Solomon.* I would like to comment on the question that Denton raised. 20$\alpha$-Dihydroprogesterone, it was almost 10 years ago we worked about this problem, was formed in any tissue of the fetus including the fetal thyroid. Diczfalusy found in placenta, *in situ* perfusion studies, that 20$\alpha$-dihydroprogesterone in fetal tissues goes back to the placenta and is re-cycled to form progesterone.