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Maternal Serum 11-Hydroxycorticosteroids during Normal Pregnancy and Abnormal Pregnancy Particularly in Cases of Anencephalus

(serum 11-OHCS/anencephalus/fetal adrenal)

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To investigate the endocrinological relationship between the mother and fetus during pregnancy, endocrinological specificity, particularly the function of the fetal adrenal gland and the placenta, synthesis and metabolism of maternal corticosteroids, we determined maternal serum 11-OHCS in normal and abnormal pregnancy including cases of anencephalus.

The maternal serum 11-OHCS levels in normal pregnancy increased gradually from the 8th week to the terminal stage of pregnancy, were about 2.5 times that of normal nonpregnant women and decreased significantly four weeks after delivery compared with the 10th month of pregnancy.

The diurnal variations in serum 11-OHCS of women in the 10th month of normal pregnancy was almost the same as that of normal nonpregnant women, with high levels observed at 6—10 a. m. and low levels in the afternoon.

The maternal 11-OHCS in abnormal pregnancy showed significantly low levels in abnormal pregnancy with intrauterine fetal death and anencephalus compared with normal pregnant women, and high levels in mild cases and low levels in severe cases were often seen in toxemia pregnancies in the later stage of pregnancy. High levels were also evident in many cases of threatened abortion and premature labor.

The maternal serum 11-OHCS levels of normal pregnancy during labor increased up to the time of delivery and showed a marked decrease thereafter.

Thus, it is assumed that the synthesis of maternal corticosteroids is concerned with function of the fetal adrenal glands and placenta.

It is mainly the fetus that plays a leading role in the endocrinological specificity of the pregnant women.

Protein hormones such as HCG and HPL of which production is increased markedly during pregnancy as well as steroid hormones including estrogen and progesterone are all produced mainly by the fetal side with the fetoplacental system as an axis.

That is, the mother is rather in a passive position endocrinologically, and a specific environment is formed in accordance with hormones produced on the fetal side.

Bryans and Belither (1) reported that maternal blood corticosteroids (cds) levels during pregnancy increased gradually as the number of pregnant weeks increased.

Murphy and Diez (2), Smith and Sherman (3, 4) held that the adrenal function of the fetus is concerned with the maturity of the fetus and onset of labor pain.

Takagi (5) determined maternal, umbilical arterial, venous blood 11-hydroxycorticosteroids (11-OHCS) levels at delivery and reported that 11-OHCS levels are higher in a mode of delivery which is accompanied by greater stress.

We attempted to determine the correlation between maternal blood cds levels and function of the fetoplacental system during pregnancy, the authors determined serum 11-OHCS levels according to the number of pregnant months in normal pregnant women, and serum 11-OHCS in abnormal pregnant women centering around those with anencephalus. Relationship between the mother and the fetus was also investigated from this aspect.

MATERIALS AND METHODS

The subjects consisted of 8 normal nonpregnant women aged 21-25 yr, 341 normal pregnant women and puerperal women plus abnormal cases including threatened abortion 44, threatened premature labor 25, hydatidiform mole 6, intrauterine fetal death 7, anencephalic pregnancy 13, multiple pregnancy 4, and toxemic pregnancy in the later stage of pregnancy 44 cases (33 mild, 11 severe).

Collection of Blood and Urine in Pregnant Women

With pregnant women, 3 — 5ml of blood were collected from the vena cubiti at around 10 a. m. and after centrifugation, the sera were frozen to —10°C for storage until determination of 11-OHCS.

For pregnant women with fetal death and anencephalus, estrogen was determined in the 24-hour urine sample.

A Study on Diurnal Variations in Serum 11-OHCS Levels in Pregnant Women

Blood was collected on six occasions, namely, at 2 a. m., 6 a. m.,10 a. m., 2 p. m., 6 p. m. and 10 p. m. in five hospitalized pregnant women in the 10th month of normal pregnancy.

A Study during the Course of Delivery

In five cases of vaginal normal delivery, blood was collected on admission, when the cervical os was fully dilated, immediately after delivery and 24 hr after delivery for determination of maternal serum 11-OHCS levels.

A Study in Puerperal Women

Serum 11-OHCS levels were determined in puerperal women who showed no particular clinical findings at the physical examination done four weeks after delivery.

Determination of Serum 11-OHCS

Serum 11-OHCS was determined by Usui's method (6, 7), a modification of

De Moor's original method (fluorimetric determination method).

Determination of Urinary Estrogen

Urinary estrogen was determined using the Amberlite XAD-2, E_3 Kit (Teikoku Zoki K. K.).

RESULTS

1. Serum 11-OHCS in Normal Nonpregnant Women

Serum 11-OHCS levels in normal nonpregnant women (21 — 25 yr of age) were 10.8 — 22.5 μg/dl, the mean value being 16.2 ± 36μg/dl.

2. Variations in Serum 11-OHCS at Each Period of Normal Pregnancy and at 4 Weeks of Puerperium

Maternal serum 11-OHCS was determined and studied for each period of pregnancy and one month of puerperium in a total of 336 normal pregnant

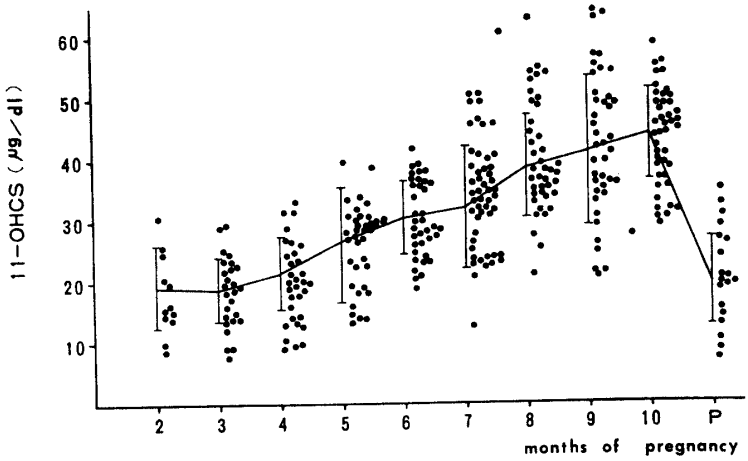

Fig. 1. Variations in maternal serum 11-OHCS concentration throughout months of pregnancy. P : 4 weeks postpartum.

women and normal puerperal women and results are shown in Fig. 1. The mean value was 18.3 ± 5.9 μg/dl at 8 — 11 weeks' gestation, increased gradually with the advance of gestation, rose to 44.1 ± 7.9 μg/dl after 36 weeks' gestation and fell off four weeks after delivery to 20.8 ± 7.4 μg/dl, low values compared with the level at the 10th month of pregnancy ($P < 0.05$).

3. Successive Variations of Serum 11-OHCS at Each Period of Pregnancy and at 4 Weeks of Puerperium in the Same Normal Pregnant Women

Serum 11-OHCS was determined at each period of pregnancy in five pregnant women who showed no abnormality clinically from the third month of pregnancy until four weeks after delivery, and results are presented in Fig. 2.

The maternal blood 11-OHCS levels increased gradually with an increase in the number of gestational months and showed a decrease four weeks after delivery compared with the level at the 10th month of pregnancy ($P < 0.05$).

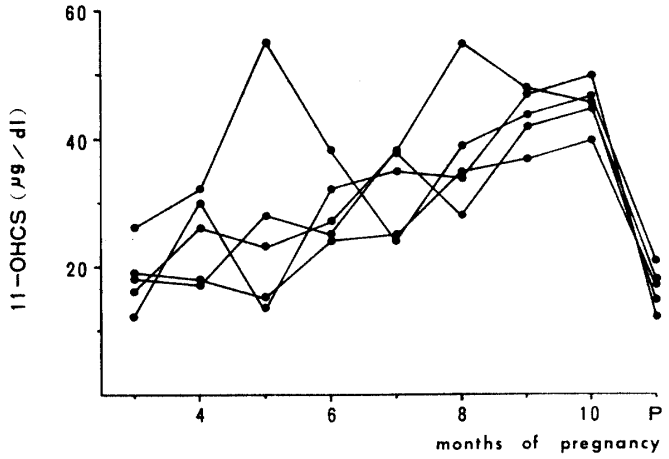

Fig. 2. Successive variations of serum 11-OHCS throughout pregnancy in the same normal pregnant women. P : 4 weeks postpartum.

However, it varied considerably from one case to another, with some cases showing high levels in the early stage of pregnancy. On the whole, the maternal 11-OHCS levels tended to increase with the advance of pregnancy.

4. Diurnal Variations of Maternal Serum 11-OHCS in Normal Pregnant Women (36 — 39 weeks' gestation)

Serum 11-OHCS of normal pregnant women (5 cases) in 36 — 39 weeks' gestation was determined on six occasions, namely at 2 a. m., 6 a. m., 10 a. m., 2 p. m., 6 p. m. and 10 p. m.

The highest level was shown at 6 a. m. in two cases and at 10 a. m. in three cases. The mean was 28.0 ± 6.2 μg/dl, the lowest at 2 a. m. and 42. 2 ± 4.6 μg/dl, the highest at 10 a. m. The five cases all showed a relatively constant level (Fig. 3).

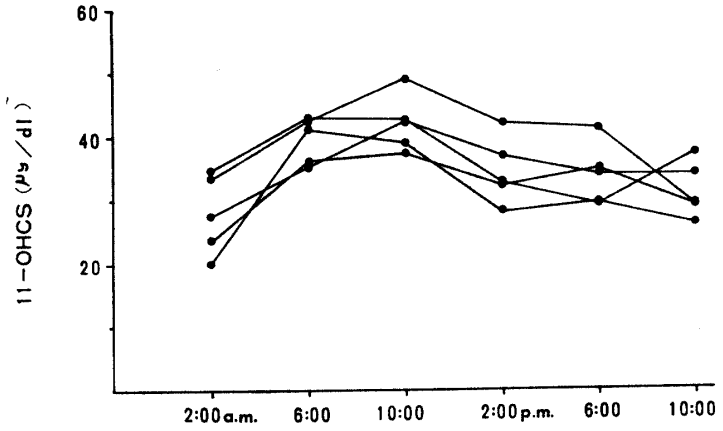

Fig. 3. Diurnal variations of maternal serum 11-OHCS concentration in normal pregnant women (36 — 39 weeks).

5. Maternal Serum 11-OHCS in Abnormal Pregnancy

a) Intrauterine fetal death

In seven with a diagnosis of intrauterine fetal death established by the disappearance of fetal movement, negative heart beat by ultrasonic doppler method and low levels of urinary estrogen, maternal 11-OHCS in six cases except one case of fetal death at the eighth month of pregnancy showed levels lower than the mean value minus SD (standard deviation) in normal pregnant women (Fig. 4).

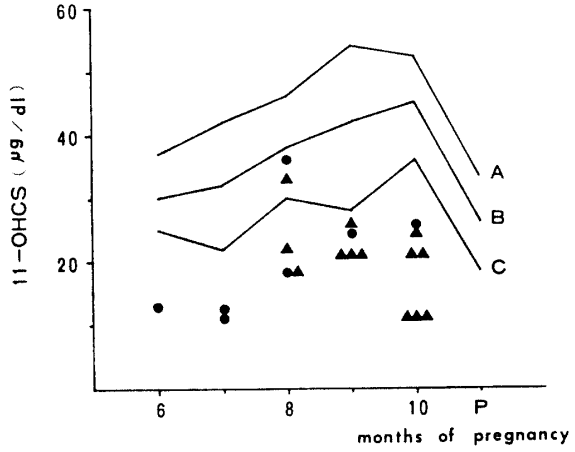

Fig. 4. Maternal serum 11-OHCS levels in cases of pregnant women with intrauterine fetal death and anencephalus. ● : intrauterine fetal death, ▲ : anencephalus, P : 4 weeks postpartum, A, B and C : $\bar{X}$ + SD, $\bar{X}$ and $\bar{X}$−SD respectively in normal pregnant women

b) Anencephalic pregnancy

Maternal serum 11-OHCS was determined in 13 cases which were diagnosed as having anencephalus by abdominal roentgenogram, the values determined of maternal urinary estrogen and clinical findings. Twelve out of the 13 showed levels lower than the mean value minus SD in normal pregnant women as illustrated in Fig. 4.

Anencephalus was confirmed by autopsy after delivery in all of these 13 cases.

c) Toxemic pregnancy in the later stage of pregnancy

The maternal 11-OHCS level in 33 mild ceses was 55.0 ± 21.6 μg/dl, and many showed levels higher than the mean of normal pregnant women, but there was no significant difference.

In severe cases, the maternal 11-OHCS level was 33.7 ± 11.3 μg/dl. They tended to show levels lower than the mean value compared with normal pregnant women (Fig. 5).

d) Twin pregnancy

In four diagnosed as twin pregnancy by roentgenogram of the abdomen before delivery, the maternal 11-OHCS level was higher than the mean value

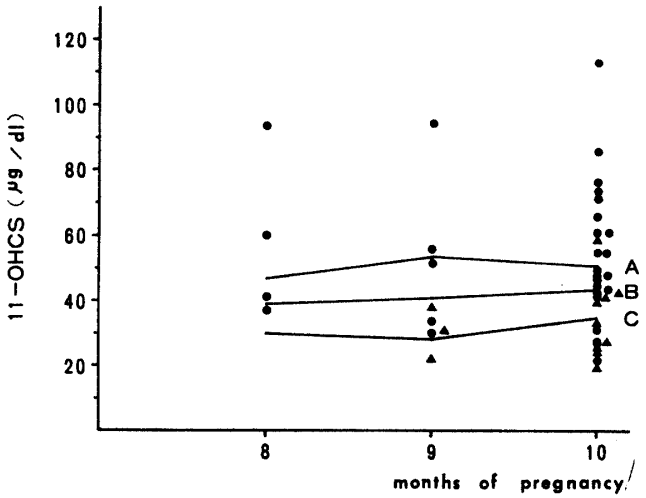

Fig. 5. Maternal serum 11-OHCS levels in toxemia of late pregnancy. ● : mild toxemia, ▲ : severe toxemia, A, B and C : same as Fig. 4.

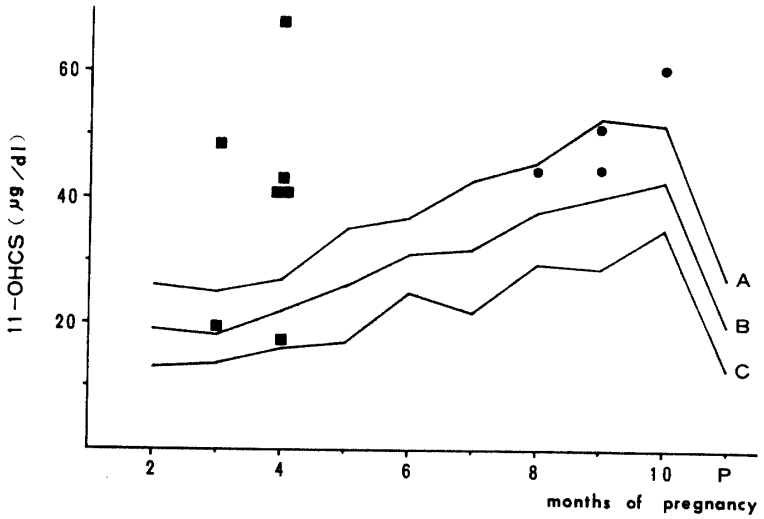

Fig. 6. Serum 11-OHCS levels in pregnant women with twins and hydatidiform mole. ● : twin, ■ : hydatidiform mole, A, B and C : same as in Fig. 4.

of normal pregnant women with 3 cases showing levels within the limits of the mean value ± SD and 1 case a considerably high level. The mean value for the 4 cases was 51.9 ± 9.0 μg/dl (Fig. 6).

e) Hydatidiform mole

In seven cases of hydatidiform mole, the maternal 11-OHCS level was considerably higher than the mean value ± SD of normal pregnant women in five cases and was within the limits of the mean value ± SD in two cases (Fig. 6).

f) Threatened abortion and premature labor

Maternal 11-OHCS was determined in 25 cases of threatened abortion and premature labor. It tended to show high levels in the former compared with normal pregnancy.

In the latter, the more serious the clinical symptoms, the higher were the maternal 11-OHCS levels (Fig. 7).

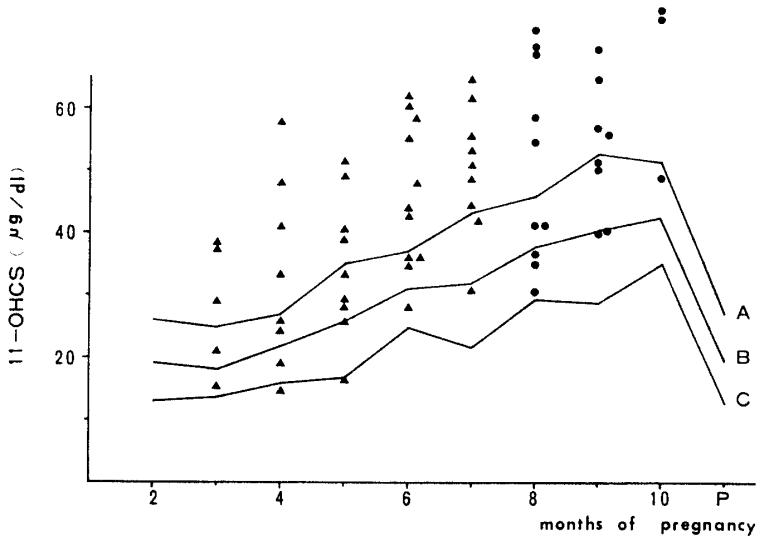

Fig. 7. Maternal serum 11-OHCS levels in cases of threatened abortion and premature labor during pregnancy. ▲ : threatened abortion, ● : threatened premature labor, A, B and C : same as in Fig. 4.

DISCUSSION

Blood 11-OHCS has the chemical structure of 11 β-hydroxy Δ^4-3-oxosteroid and consists of cortisol and a minute amount of corticosterone.

Cortisol is the main substance of glucocorticoids secreted from the adrenal cortex. The bulk of blood cortisol is combined with protein and shows no biological activity. Free cortisol not combined with protein reportedly shows the biological activity (8).

Serum 11-OHCS determined in the present study is the sum total of the free type and the protein combined type.

We used the relatively easy-to-determine serum total 11-OHCS in this study.

Determination of serum 11-OHCS was made according to Usui's modification of De Moor's method.

When the diurnal variations in the maternal serum 11-OHCS levels in normal pregnant women in the 10th month of pregnancy were examined, we found that the maternal serum 11-OHCS showed the highest level at 6 a. m. — 10 a. m. and that the levels decreased during the night.

Usui and Kawamoto (6, 9) reported that in determining the normal value the level at 10 a. m. was made the standard so that it can be applied to out-

patients since diurnal variations in blood 11-OHCS were extreme.

In our work herein, we collected blood from pregnant women at 10 a. m. and referred to it as the standard sample.

According to results of the present study, the mean value of 11-OHCS was 16.2 ± 3.6 μg/dl in healthy nonpregnant women, 18.3 ± 5.9 μg/dl in pregnant women at the third month of pregnancy, increased gradually with an increase in the number of gestational months and reached 44.1 ± 7.9 μg/dl at the 10th month of pregnancy, about 2.5 times that in the early stage of pregnancy.

This is consistent with the finding reported by Friedman (10) that blood cds increased with the advance of pregnancy.

Regarding the reason for an increase in blood cds during gestation, Smith (11) reported that the weight of adrenal cortex in pregnant monkeys increased to about double the level shown during nonpregnancy.

Akasu (12) held that cholesterol in the adrenal cortex of pregnant white rats decreased compared with levels during nonpregnancy and that the degree of decrease was higher in the early stage than in the later stage of pregnancy.

Thus, it was considered that maternal blood cds would increase as the maternal adrenocortical function was enhanced during pregnancy.

However, Whitely and Stoner (13) found no increase in the weight of the adrenal cortex in pregnant women who died suddenly. In a comparative study of the histological changes in the adrenal cortex during pregnancy and those in Cushing's syndrome, they stated it was difficult to explain the increase in blood cds of pregnant women with reference to the adrenal cortex.

Meanwhile, there is a report (12) in which the authors attributed the increase in blood cds in pregnant women to the placenta and extracted a cds-like substance.

However, Matsuba (14) by incubating the human placenta and Higuchi (15) in experiments with placental perfusion reported that cortisol was not produced in the placenta.

Referring to the cds production in the fetal adrenal gland, Miglon *et al.*(16) considered cds in the fetus to be derived from the mother on the grounds that when radioactive progesterone or corticol is administered via vena cubiti to women in the terminal stage of pregnancy, it moves quickly to the fetal side.

However, Block and Benirechke (17) reported that the adrenal gland of human fetuses would have the ability to synthesize cds. When ^{14}C-labeled acetic acid was incubated with slices of the fetal adrenal gland, radioactive cortisol was obtained. Terazawa (18) reported that the adrenal tissue of the fetus in the 5th — 7th month of pregnancy had a marked reaction on ACTH in vitro to yield cortisol. Hilman and Giroud (19) stated that the neonatus immediately after delivery would adapt themselves to the living environment through the exercise of their own adrenocortical function.

Takagi (5) also found the cds production by the fetus itself on the basis of

the difference in the levels of umbilical arterial and venous blood 11-OHCS during labor.

Beitins *et al.* (20) reported that the placenta regulated the movement of cds between the mother and fetus, by which cortisol secreted of the fetal side moved easily to the maternal side but that in the reverse case, the placenta served as a barrier to bring about hemostasis which maintained the fetal cortisol level constant.

Thus, it is assumed that the fetus itself has an independent adrenocortical function and that cortisol secreted from the adrenal gland of the fetus will be introduced from the umbilical artery through the placenta into the mother and thereby contribute to the increase in maternal blood cds. The fetus-originated cortisol probably is, to some extent, mixed with the cortisol in the maternal blood.

Under the influence of estrogen the levels of which increase markedly during pregnancy, maternal blood cortisol binding globulin (CBG) increases and subsequently the binding ability of cds increases. However, it is said that the protein binding type of cds is not easily metabolized and is not readily excreted from the kidney (21) and is pooled in blood.

If indeed the cds production by the fetal adrenal gland is related to the amount of cds in the maternal blood, a fall in maternal blood cds of intrauterine fetal death naturally occurs.

Maternal estrogen is also decreased due to the DHEA sulfate secretion from the adrenal gland of the fetus and the 16-α-hydroxylation activity of the fetal liver being impaired.

The reason for the low level of maternal blood 11-OHCS could also be explained by the participation of estrogen in the cds metabolism during pregnancy.

The same can be said of pregnant women with anencephalus. The maternal blood 11-OHCS level is presumed to be low compared with normal pregnant women in view of such factors as the fall on DHEA sulfate secretion due to declined adrenal function of the fetus, subsequent marked decrease in production of estrogen and further a decrease of the cds production.

Judging from the above, it appears quite possible to estimate the amount of maternal urinary estrogen and the function of the fetoplacental system including the placental and adrenal function by determination of maternal serum 11-OHCS and blood CBG.

Assali *et al.* (22) and Friedman (10) maintained that the maternal blood cds level in pregnant women with toxemia in the latter stage of pregnancy was the same as that in normal pregnant women.

However, Murkerjee and Swyer (23) reported that the blood cds level in this disease was higher than that in normal pregnancy. In the present study, the blood cds level was somewhat higher in mild cases but there was no significant difference compared with levels during normal pregnancy, while in severe cases many showed slightly low levels compared with those estimated during normal pregnancy.

As for serum 11-OHCS in threatened abortion and premature labor, the more serious the clinical symptoms, the higher was the level in many cases compared with normal pregnancy.

Kitao *et al.* (24) reported that the blood 11-OHCS level in pregnant women who were hospitalized with labor pain was about 20 μg/dl higher than that in the 10th month of pregnancy. Symptoms such as abdominal pain and labor pain appear to increase free cortisol in the mother.

Takagi (5) reported that the maternal and umbilical blood 11-OHCS level was higher in modes of delivery which placed greater stress on the fetus.

As mentioned earlier on, fetal 11-OHCS is considered to be partly mixed with maternal blood and from these findings it is assumed that high levels of maternal blood 11-OHCS will be due to mixed cds of maternal and fetal origins which are increased with stress.

From the result of the present study and various reports mentioned above, the increase in maternal blood cds during normal pregnancy is apparently not due to free cds but to an increase in CBG-bound cds.

As the siginificance of an increase in the CBG-bound cds, the following may be deduced.

The CBG-bound cds capable of transporting hormone in blood prevents hormone from readily entering the cells and when hormone secretion decreases rapidly for one reason or another, it becomes free cds to make up for the decrease in hormone.

Furthermore, the protein binds to hormones in which the molecules are small and these protein binding hormones are not readily excreted into urine but rather are accumulated in blood to create such a hormone environment that pregnant women themselves can cope with preparation for labor, stress and emergency.

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