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Free Estrone, Free Estradiol, Free Estriol, Free Estetrol, Total Estriol and Dehydroepiandrosterone Sulfate in Mother and Fetus at Delivery

(fractions of estrogen/DHA-S/maternal and umbilical blood/delivery)

FUMINORI MURAO[a], KIYOSHI HASEGAWA[b], KENICHI KANEDA[a], ISAO MATSUNAGA[a], and MANABU KITAO[a]

[a]*Department of Obstetrics and Gynecology, Shimane Medical University, Izumo 693 and*
[b]*Department of Obstetrics and Gynecology, Matsue Red Cross Hospital, Matsue 690, Japan*

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To elucidate estrogen related factors in the mother and fetus at the time of expulsion, maternal blood and umbilical arteriovenous blood samples were collected from the same women and F-E_1, F-E_2, F-E_3, F-E_4 and T-E_3 were determined simultaneously by RIA DHA-S, using the acid fluorescence reaction.

The 4-fractions of free estrogen in the maternalblood were high in the order of F-E_1>F-E_2>F-E_3>F-E_4, and for umbilical arteriovenous blood, the order was F-E_3>F-E_2>F-E_1>F-E_4.

The dehydroepiandrosterone sulfate level, as the precursor of estrogen, was higher in umbilical arteriovenous blood than in maternal peripheral blood, therefore, the dehydroepiandrosterone sulfate is probably excessively secreted in the fetal adrenals.

These results suggest that there is a difference in the production pattern and the metabolism in each fraction of these estrogens.

The free estetrol in the maternal blood is assumed to be derived from the feto-placental system, considering the production pathway, as the free estetrol level was higher in the umbilical arteriovenous blood than in the maternal blood.

Estrogen in the maternal blood during pregnancy differs in production and metabolism from that seen in nonpregnant women.

Characteristics of estrogen have been clarified by Diczfalusy and Mancuso (1) and there have been several reports on the behavior of estrogen in maternal blood and umbilical blood, at the time of delivery.

Clinically, estrogen in the maternal blood and urine is determined as an index for the function of the fetoplacental system, since estrogen during pregnancy is produced in the feto-placental system and increases markedly with the advance of pregnancy.

Maternal blood estrogen is classified into several tens of kinds during the course of metabolism, each of which is detected as a free type and a conjugated type.

We collected maternal blood and umbilical arteriovenous blood from the same women at the time of expulsion of the fetus, and determined free estrogen (F-E_1), estradiol (F-E_2), estriol (F-E_3), estetrol (F-E_4), total estriol (T-E_3), simultaneously by RIA dehydroepiandrosterone sulfate (DHA-S) using the acid fluorescence reaction (2), and then studied the related behavior.

SUBJECTS AND METHODS

The subjects were ten Japanese women with spontaneous onset of labor pains followed by transvaginal delivery.

Immediately after expulsion of the fetus, maternal peripheral blood (MPV) from the same women and umbilical arterial blood (UA) and umbilical venous blood (UV) from the neonates were collected.

The blood was centrifuged to separate the serum and then was stored at—20°C until various determinations.

Serum F-E_1, F-E_2, F-E_3 and F-E_4 were determined with CIS and F-E_3 (Green Cross) and T-E_3 with Kaken Kagaku make RCC. Serum DHA-S was determined by the acid fluoresence reaction (2).

The rejection limit method was used for statistical treatment and the "t" test for calculation of significant difference.

RESULTS

1. Maternal Blood Estrogen Levels at Delivery

Maternal blood estrogen levels at normal delivery are shown in Table I.

The concentration for 4 fractions of estrogen was highest with F-E_2, showed no significant difference between F-E_1 and F-E_3 and was lowest with F-E_4. (Table I)

Though the subjects and the method of determination differed, the concen-

TABLE I. *Serum Free Estrone, Estradiol, Estriol and Esthetrol Concentration in Paired Maternal Peripheral Blood and Umbilical Vein and Artery Specimens*

	Serum free estrone		Serum free estradiol			Serum free estriol			Serum free esthetrol		
	M.P.V (ng/ml)	U.V (ng/ml)	M.P.V (ng/ml)	U.V (ng/ml)	U.A (ng/ml)	M.P.V (ng/ml)	U.V (ng/ml)	U.A (ng/ml)	M.P.V (ng/ml)	U.V (ng/ml)	U.A (ng/ml)
S.A	16.9	3.7	50.2	13.6	15.3	28.1	150.3	91.1	1.03	3.80	2.85
S.S	6.3	0.8	34.5	4.8	9.2	23.1	46.8	-	1.22	3.14	3.82
N.Y	17.2	8.9	33.0	20.6	14.4	22.1	111.3	63.7	1.03	4.03	3.82
H.W	5.4	4.2	37.8	21.4	17.3	20.9	127.5	111.7	0.51	2.51	2.81
N.Y	16.4	1.1	14.8	5.0	1.6	8.6	56.6	34.6	0.34	2.66	-
S.H	55.2	10.7	30.9	15.3	5.0	12.0	88.8	39.0	0.44	3.66	1.73
I.M	14.2	5.6	43.2	5.8	4.7	22.5	54.7	55.1	0.66	2.28	1.22
S.M	12.2	5.7	19.1	28.1	15.0	15.7	126.7	100.8	0.48	4.91	3.87
M.I	14.5	11.5	19.2	-	4.2	13.8	-	57.3	1.06	-	2.90
M.K	20.7	3.1	47.2	16.6	6.0	16.8	71.6	40.1	0.88	3.38	3.31
M ± SD	17.9±13.9	5.5±3.7	33.0±11.6	14.6±7.7	9.3±5.4	17.8±5.3	91.2±33.7	72.9±33.0	0.81±0.27	3.46±0.79	3.46±0.79

M. P. V : maternal peripheral vein, U. V : umbilical vein, U. A : umbilical artery
Abbreviations are the same for all Tables.

tration for maternal blood F-E_3 and T-E_3 was also evaluated. (Table II)

The level for maternal blood T-E_3 was about six times that for F-E_3, there being a correlation between T-E_3 and F-E_3 ($r=0.7$, $0.01<P<0.02$). (Fig. 1)

TABLE II. *Serum Total Estriol and Free Estriol Concentrations in Paired, Maternal Blood, Umbilical Vein and Umbilical Artery Specimens*

	Total estriol ng/ml			Free estriol ng/ml		
	M. P. V	U. V	U. A	M. P. V	U. V	U. A
Y. U	335	3080	2970	61.5	244	102
K. K	120	—	2150	34.0	518	216
N. N	410	2650	2715	37.0	247	100
K. K	130	1450	1595	18.5	137	67
Y. Y	75	1270	1245	18.0	190	64
S. Y	135	1960	1920	36.5	274	97
T. T	90	1980	1812	27.5	187	81
H. M	295	1880	1945	34.5	193	154
F. K	412	2255	2320	53.0	218	127
K. Y	290	1605	1675	43.0	140	93
M±SD	229±126	2014±543	2034±494	36.3±13.0	203±44	110±43

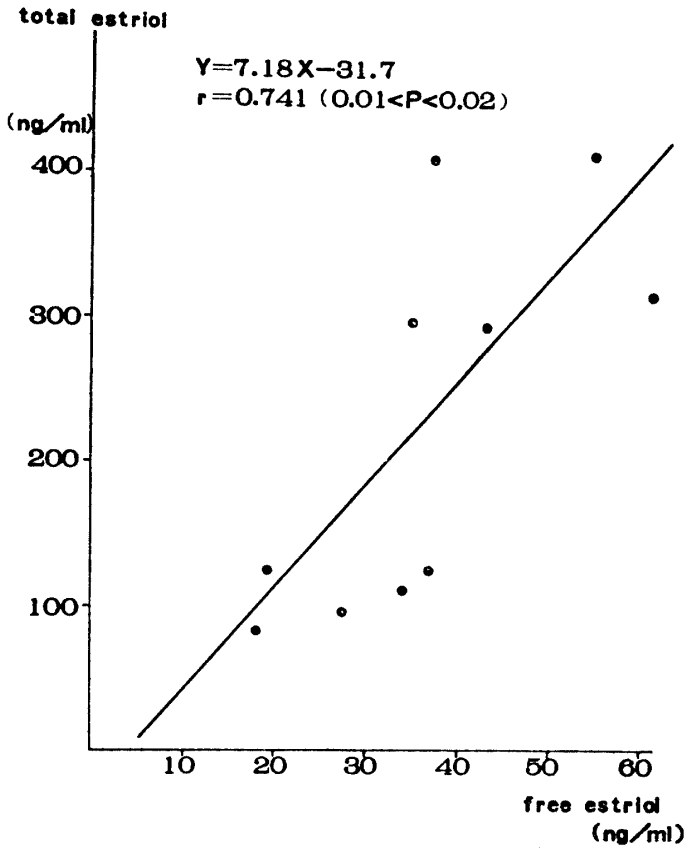

Fig. 1. Serum total estriol and free estriol concentration in maternal peripheral blood.

2. Estrogen Levels in Umbilical Arteriovenous Blood

The concentration for 4 fractions of estrogen was highest with F-E_3 and lowest with F-E_4. (Table I) The level of T-E_3 in the umbilical arterial blood was about 18 times that of F-E_3. The concentration for 4 fractions of estrogen was highest with F-E_3 and lowest with F-E_4, as in the umbilical arterial blood. (Table I) The level for T-E_3 in the umbilical venous blood was about ten times that for F-E_3. (Table II)

3. Comparison of Maternal Blood Estrogen Levels and Umbilical Arterial Blood Estrogen Levels

The maternal blood F-E_2 showed higher levels than the umbilical arterial blood F-E_2, with 33.0 ± 11.6 ng/ml for the former and 9.3 ± 5.4 ng/ml for the latter ($P<0.001$). The F-E_3 level was lower in maternal blood than in umbilical arterial blood, the maternal blood T-E_3 level was lower than the umbilical arterial blood T-E_3 level and there was a correlation between the concentration of the two ($r=0.7$, $0.01<P<0.02$). (Fig. 2, Table II)

The maternal blood F-E_4 level was lower than the umbilical arterial blood F-E_4 level.

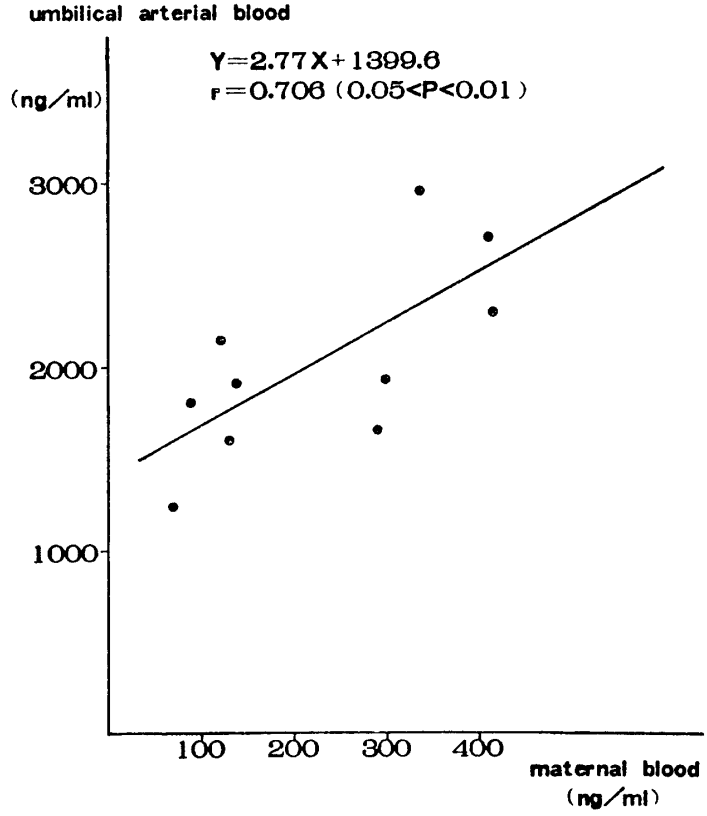

Fig. 2. Serum total estriol in maternal blood and umbilical arterial blood.

4. Comparison of Maternal Blood Estrogen Levels and Umbilical Venous Blood Estrogen Levels

The maternal blood F-E_1 level was higher than the umbilical venous blood F-E_1 level, the maternal blood F-E_2 level was higher than the umbilical venous blood F-E_2, the maternal blood F-E_3 level was lower than the umbilical venous blood F-E_3 level and the maternal blood T-E_3 level was lower than the umbilical venous blood T-E_3 level.

5. Comparison of Umbilical Arterial Blood Estrogen Level and Umbilical Venous Blood Estrogen Level

The umbilical arterial blood F-E_2 level was lower than the umbilical venous blood F-E_2 level, the umbilical arterial blood F-E_3 level was lower than the umbilical venous blood F-E_3 level and the umbilical arterial blood F-E_4 level was lower than the umbilical venous blood F E_4 level.

There was no significant difference between the umbilical arterial blood T-E_3 and the umbilical venous blood T-E_3 level, but there was a correlation in the concentration ($r=0.9$, $P<0.001$). (Fig. 3)

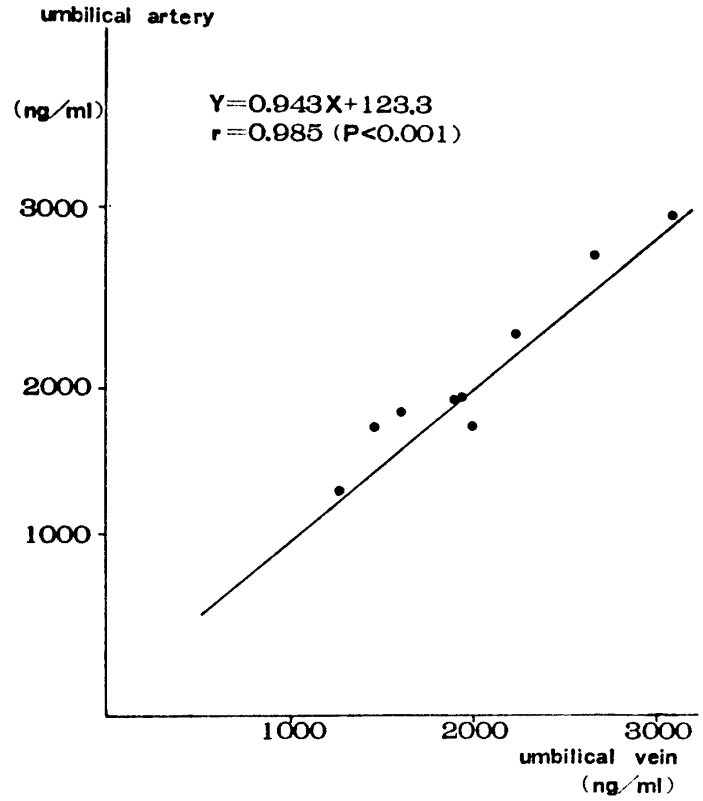

Fig. 3. Total estriol in umbilical vein and artery specimens.

6. F-E_3/T-E_3 Ratio in Maternal Blood and Umbilical Arteriovenous Blood

A study was made of the concentration ratio of F-E_3 and T-E_3 in the maternal blood, umbilical arterial blood and umbilical venous blood.

The concentration ratio of F-E_3 to T-E_3 was high in the order of the

TABLE III. *Serum Free E_3/Total E_3 Ratio in Paired, Maternal Blood, Umbilical Vein and Umbilical Artery Specimens*

	Free E_3/total E_3 ratio (%)		
	N. P. V	U. V	U. A
Y. U	18	8	3
K. K	28	—	10
N. N	9	9	4
K. K	14	9	4
Y. Y	24	15	5
S. Y	27	14	5
T. T	31	9	4
H. M	12	10	8
F. K	13	10	5
K. Y	15	9	6
M±SD	19±7	10±2	5±2

maternal blood>umbilical venous blood>umbilical arterial blood ($P<0.002$). (Table III)

7. Comparison of Maternal Blood DHA-S Levels and Umbilical Arterious and Venous Specimens

The level for DHA-S in the umbilical arterious blood was higher than levels for DHA-S in both the umbilical venous blood and the maternal blood. (Table IV)

TABLE IV. *Serum DHA-S Concentrations in Paired, Maternal Peripheral Blood, Umbilical Vein and Umbilical Artery Specimens*

	DHA-S ug/ml		
	M. P. V	U. V	U. A
T. Y	0.31	0.86	0.72
M. I	0.18	0.73	0.78
K. H	0.25	0.73	0.79
I. S	0.21	0.78	1.16
M. T	0.18	0.74	0.83
Y. H	0.19	0.88	0.95
S. S	0.25	0.94	0.99
U. K	0.22	0.60	0.69
Y. S	0.25	0.62	0.65
M±SD	0.23±0.04	0.76±0.11	0.84±0.16

DISCUSSION

There are few reports on the simultaneous determination of E-F_1, F-E_2, F-E_3, F-E_4 and T-E_3 using the same sample in the maternal blood and umbilical arteriovenous blood at delivery. The F-E_1, F-E_2, F-E_3 and total estriol levels, as determined by our team in the maternal blood and umbilical arteriovenous blood at fetal expulsion were similar to those reported by Yoshida (3) and the F-E_4 level was lower than that reported by Tulchinsky *et al.* (4).

In a comparison of the estrogen fractions in the maternal blood, umbilical arterial blood and umbilical venous blood, it was assumed that the pattern of estrogen production in the feto-placento-maternal system would vary with the estrogen and that the metabolic pathway would differ from one estrogen to another.

Androgens from either the mother or the adrenal gland of the fetus are directly concerned with the estrogen production in the feto-placento-maternal system, as precursors. Here, DHA originating from the adrenal gland of the fetus plays a leading role and is broken down in the placenta for conversion into estrone and estradiol and then it undergoes 16α-hydroxylation in the liver of the mother for conversion into estriol.

Since, however, the 16α-hydroxylation activity of the mother is low, the conversion to estriol in the placentomaternal system is said to be low (5).

In the placenta, C_{19} steroid is readily converted to estrone and estradiol but cannot be converted to estriol since there is no 16α-hydroxylase in the placenta. However, 16α-hydroxylated C_{19} steroid can be converted to estriol. The concentrations of 4 fractions of estrogen in the maternal blood were similar to those reported by of Seo *et al.* (6). F-E_1 and F-E_2, produced in the placenta were directed to the mother and to the fetus. In this case, the rate of movement to the mother appears to be predominant.

From the data that the estrone and estradiol levels in pregnant women with Addison's disease are lower than those in pregnant women with an ancephalus fetus, it is assumed that the adrenal gland of the mother is concerned with production of maternal blood estrone and estradiol (7).

The concentrations in 4 fractions of the umbilical arterial blood estrogen, were high in the order of F-E_3>F-E_2>F-E_4. The F-E_3 level in the umbilical arterial blood was four times as high as that in the maternal blood.

These results are consistent with findings regarding the production and metabolism of estrogen in the fetoplacental system.

Our observations suggest that maternal blood estriol originates from the feto-placental system. The F-E_4 level was low compared with that reported by Tulckinsky *et al.* (4), but the concentration was similar for both the maternal and umbilical arteriovenous blood.

The maternal blood and umbilical arteriovenous blood F-E_4 showed the lowest level among the 4 fractions of estrogen determined in this study.

For estetrol, 80 percent of the precursors originate from the fetus ; 16α-hydroxylated C_{19} steroid from the fetus is subjected to aromatization in the placenta for conversion to estradiol and estriol which in turn are subjected to 15α-hydroxylation in the liver and the adrenal gland of the fetus for conversion to estetrol (8).

The concentration of F-E_4 in maternal blood and umbilical arterial blood is similar to that of F-E_3. The umbilical arterial blood F-E_4 level was about three times as high as the maternal blood level, and umbilical venous blood F-E_4 showed higher levels than umbilical arterial blood F-E_4.

There was no correlation of concentrations between maternal blood F-E_4 and umbilical arterial blood and between umbilical arterial blood and umbilical venous blood, but the umbilical arterial blood F-E_4 level was higher than the maternal blood level. From the route of F-E_4 production, it was assumed that maternal blood F-E_4 originates in the feto-placental system.

Estrogen in the maternal blood and umbilical arteriovenous blood is present as a free or conjugated type, and the free type exerts a biological action.

In the present study, F-E_3 and the total estriol (T-E_3) were determined and studied in the same sample.

The maternal blood F-E_3/T-E_3 ratio was higher than the umbilical arterial blood F-E_3/T-E_3 ratio. The proportion of F-E_3 was higher in maternal blood than in fetal blood.

F-E_3 levels were higher in umbilical venous blood than in umbilical arterial blood, and there was no significant difference in T-E_3 between umbilical arterial blood and umbilical venous blood, suggesting that a regulatory mechanism for the conjugation of F-E_3 exists in the fetus.

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