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SEX DIFFERENCES IN FETAL GONADOTROPINS AND ANDROGENS

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SUMMARY

We investigated the hypothalamo-hypophyseo-gonadal system in human fetuses and neonates by serially sampling fetuses obtained either during spontaneous abortions, premature deliveries or under the conditions of the Eugenics Protection Law. Maternal specimens were obtained serially throughout pregnancy, at parturition and in the puerperium. The purpose of our study was to determine the presence or absence of sex differences in fetuses and neonates by determining plasma constituents. The subjects for determination were FSH, LH, hCG and its β-subunit, and androgens. We also attempted to determine the effects of LH-RH priming on the anterior hypophysics. We found fetal sex differences in FSH and testosterone levels, but were unable to detect any differences in the other materials determined. Furthermore we were able to detect an anterior pituitary response to LH-RH priming from the second trimester of pregnancy.

INTRODUCTION

In recent years, the endocrinological investigation by Jost[1] in animals and those by Grumbach and Kaplan[2, 3] on the histochemical and clinical significance of the fetal hypophysis and evaluations of fetal endocrine function have resulted in advances in the understanding of the hypothalamo-hypophyseogonadal system. These various approaches have revealed the existence of a stimulatory–inhibitory relationship in the fetal hypothalamo-hypophyseogonadal system extant during intrauterine development. Sexual differentiation especially the testicular androgens have been established early in fetal development. However, the specific effects of this androgenic differentiation on definitive sexual differentiation of the fetus are not yet completely understood. Further, the fetal endocrine systems differentiate and develop function in the enclosed environment of the uterus in conjunction with placental and maternal endocrine function. This investigation involves the evaluation of fetal plasma FSH, LH and other gonadotropin concentrations as in Grumbach *et al.*'s report, as well as the evaluation of the effects of LH-RH priming on fetal gonadotropin and testicular androgen levels.

MATERIALS AND METHODS

Blood samples. Blood sampled from the antecubital veins of the mothers and the umbilical arteries of fetuses of later than 8 weeks development in cases of therapeutic abortions under the conditions of the Eugenic Protection Law, spontaneous abortions, premature deliveries and term deliveries. Follow up samples were obtained from neonates serially up to 10 days and at 1 month postpartum, and 1 year old infants. All specimens were transferred to heparinized tubes immediately after sampling, centrifuged at 3500 rev./min for 5 min, and the plasma was stored at −20° until assayed.

A series of 31 subjects consisting of fetuses of more than 16 weeks development obtained through therapeutic termination of pregnancy, premature infants and normal term infants requiring replacement transfusion were primed with LH-RH 2 μg/kg intramuscularly. The controls were 11 fetuses and neonates who were administered 0.02 ml/kg physiological saline. Both the LH-RH primed subjects and the controls were serially sampled *via* the umbilical blood vessels (3 ml/sample) and all blood samples were treated as described above.

Testicles were obtained from fetuses of 4–6 lunar months development who spontaneously aborted or were therapeutically aborted, as soon as feasible following clinical death. Selected cases were primed with 5000 I.U. HCG intravenously prior to clinical death to evaluate HCG receptor sites by the fluorescent antibody method.

FSH and LH. FSH and LH were determined using Calbiochem's RIA kit, a double antibody method, using as standard 2nd-IRP-HMG. Results were recorded as mi.U./ml. The cross reaction of HCG to LH was highly concentration dependant, with high concentrations of LH in low cross reaction rates with HCG and low concentrations of LH giving high cross reaction rates.

HCG and HCG-β subunit. HCG was determined using CIS's double antibody RIA kit with results expressed in ng/ml, with 1 ng/ml equivalent to 6.6 mi.U./ml. HCG-β subunit was determined using HCG-β subunit and anti HCG-β subunit provided by NIAMD by the Vitukaitus[4] double antibody method. [^{125}I]-HCG-β subunit was separated with Sephadex G-50. The first and second incubations were highly stable at 4° for 3 days and 1 day respectively, resulting in high binding rates.

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Table 1. The cross reactivity of various steroids to the antiserum testosterone-3-oxime-BSA

Compound	% Cross reaction
Testosterone	100
5α-dihydrotestosterone	117
Epitestosterone	47.0
Androst-4-ene-3β, 17β-diol	26.1
Androst-5-ene-3β, 17β-diol	1.94
Androst-4-ene-3,17-diol	1.23
5-androstene-3α, 17β-diol	0.74
Androsterone	0.14
Dehydroepiandrosterone	<0.09
Aetiocholanolone	<0.09
Progesterone	0.19
17α-hydroxyprogesterone	<0.09
Cortisol	<0.09
Corticosterone	<0.09
Pregnenolone	<0.09
Cortisone	<0.09
Estrone	<0.09
Estradiol-17β	<0.09
Estriol	<0.09
Cholesterol	<0.09

Table 3. The cross reactivity of various steroids to the antiserum 4-ene-androstenedione-3-oxime-BSA

Compound	% Cross reaction
4-ene-androstenedione	100
5α-androstenedione	46.1
5β-androstenedione	50.7
Epiandrosterone	9.41
Etiocholanolone	7.33
Testosterone	5.43
Androsterone	4.75
Cortisone	1.71
Progesterone	1.38
Dehydroepiandrosterone	0.81
Dihydrotestosterone	0.76
Deoxycorticosterone	0.42
Dehydroepiandrosterone sulphate	0.17
17α-hydroxyprogesterone	0.12
Cortisol	0.12
Pregnenolone	<0.072
Corticosterone	<0.072
Estrone	<0.072
Estradiol	<0.072
Estriol	<0.072

The first antibody at 3.0×10^4 dilutions resulted in a binding rate of 35–40%, and our range of determinations was 1–40 ng/ml with a deviation coefficient less than 20%. Recovery rates were well within acceptable limits and the cross reactions with HCG and LH were roughly 50% and less than 5% respectively.

Testosterone. Testosterone-3-(O-carboxymethyl)-oxime-BSA was synthesized and used as antigen to innoculate rabbits. The rabbit testosterone antiserum was used in RIA. The specificity of the antiserum was checked by its cross reactivity in response to various steroids with testosterone as 100% revealed the following: 5α-dihydrotestosterone (5α-DHT) 117%; epitestosterone 47%; androst-4-ene-3, 17-diol 26.1%; the other steroids showed reactions and the corticoids and estrogens cross reacted at less than 0.1% (Table 1).

Samples were separated using a solvent of hexane–benzene–methanol (90:5:5 by vol.) and run through microcolumns packed with Sephadex LH-20. The accuracy of the method based on the standard curve as shown in Table 2, with a sensitivity of 5 pg. Controls containing less than 20 pg showed a deviation coefficient of greater than 20% indicating that the lower limits of this product to be 20 pg. Duplicate assays of given specimens showed a precision of 9.92% (range: 0.16–8.02 ng/ml $N = 22$) in the intra-assay variance and 13.5% (range; 3.24–6.54 ng/ml $N = 55$) in the interassay variance.

4-ene-Androstenedione. 4-ene androstenedione-3-oxime-BSA was synthesized as our antigen and innoculated into New Zealand white buck rabbits. The antiserum obtained had the qualities expressed in Table 3. The cross reactivity of the antiserum based on 4-ene-androstenedione as 100% was as follows: 5α- and 5β-androstenedione were 50.7% and 46.1% respectively; Epiandrosterone 9.41%; ethiocholanolone 7.33%; testosterone 5.43%; androsterone 4.75%; cortisone 1.71%; progesterone 1.38%; the other principal steroids had negligible cross reactions to the antiserum. Based on this antiserum, we developed a RIA method for 4-ene-androstenedione. The specimens were separated and treated by the techniques described for testosterone. The sensitivity of this antiserum as derived from the standard curve is 5 pg, and the accuracy of the method as described in Table 4

Table 2. Accuracy of the method

Female plasma	Steroid added (pg)	Steroid quantified: Mean (pg)	Standard deviation	Coefficient of variation (%)	Regression equation
0.1 ml	0	23.0	5.71	24.8	$Y = 0.865x + 31.3$
	20	40.0	9.20	23.0	
	50	85.2	5.37	6.30	
	100	121	9.03	7.48	
	200	212	11.1	5.24	
	500	460	20.1	4.38	

Number of each determination, 4; X = steroid added; Y = steroid quantified.

Table 4. Accuracy of the method

Sample	Steroid added (pg)	Δ^4-androstendione quantified Mean (pg)	Standard deviation	Coefficient of variation (%)	Regression equation
Water	0	6.08	1.59	26.15	$Y = 0.99x + 8.69$
0.1 ml	20	25.38	3.01	11.85	
	50	60.26	5.30	8.79	
	100	113	9.15	8.03	
	200	206	11.66	5.63	
	500	506	16.02	3.16	
Female	0	54.4	11.34	20.84	$Y = 0.97\ X + 56.07$
plasma	20	72.4	11.58	15.99	
0.05 ml	50	102	9.53	9.34	
	100	153	12.85	8.39	
	200	263	20.46	7.77	
	500	537	23.67	4.40	

Number of determinations, 5: X = steroid added; Y = androstenedione quantified.

with a deviation coefficient of greater than 20% at concentrations of less than 20 pg, making the lower limits for determination 20 pg/sample. The precision of the technique derived from duplicate assays of a given sample and comparing the intra and interassays were as follows. The intra-assay variance was 11.02% (range; 602–1896 pg/ml, $N = 31$) and the intra-assay was 14.93% (range; 716–1548 pg/mg $N = 16$).

5α-DHT. The antiserum utilized to assay testosterone was used in our determinations of 5α-DHT. Specimens were separated in a solution of hexane–benzene–methanol (90:5:5 by vol.) and passed through micro columns containing Sephadex LH-20.

The sensitivity of the method derived from the standard curve was 5 pg. The accuracy of the method is as shown in Table 5 and the lower limits of detectability was 20 pg/sample. The precision was determined by duplicate assays of given specimens, showing an interassay variance of 10.85% (range; 320 pg/ml–590 pg/ml, $N = 5$) and an intra assay variance of 15.74% (range; 360 pg/ml–510 pg/ml, $N = 5$).

Histological and fluorescent antibody evaluation of testicular tissue. Testicular were set in soft paraffin following removal from the 4% formalin preservative and sliced into 5 μ specimens under low temperature. A portion of the specimens were treated with fluorescent antibodies to study LH and HCG receptor sites.

RESULTS

I. Gonadotropin synthesis in the anterior lobes of human fetal hypophysis

1. *Fetal plasma FSH levels (Fig. 1).* Male fetal plasma FSH levels were investigated from 13 wks of development, however, FSH was undetectable in 13 wks male fetuses. At 15–16 wks plasma FSH was 5 mi.U./ml, 17–18 wks. FSH levels were 5.5 ± 4.3 mi.U./ml, and thereafter until 35–36 wks of development remained at roughly 6 mi.U./ml. Starting at 37–38 wks. FSH levels began decreasing and at term was 3.3 ± 1.3 mi.U./ml. Female fetuses at 15–16 wks showed FSH levels of 67.5 ± 10.6 mi.U./ml, which were substantially higher than that of males of the same period of development. At about 23 wks,

Table 5. Accuracy of the method

Sample	Steroid added (pg)	5α-DHT quantified Mean (pg)	Standard deviation	Coefficient of variation (%)	Regression equation
Water	0	5.88	1.84	31.29	$Y = 1.01X + 0.99$
0.5 ml	20	25.86	4.16	16.08	
	50	59.96	9.28	15.47	
	100	105.6	14.81	14.02	
	200	201.8	20.90	10.35	
	500	517.4	49.85	9.63	
Female	0	85.2	21.15	24.82	$Y = 0.99X = 87.35$
plasma	20	105.2	12.15	11.89	
0.5 ml	50	138	14.61	10.59	
	100	184.4	17.26	9.36	
	200	294.8	23.29	7.9	
	500	581	42.27	7.28	

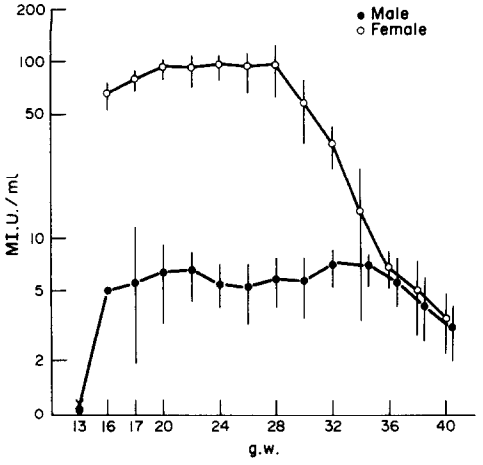

Fig. 1. Fetal plasma FSH by gestational week.

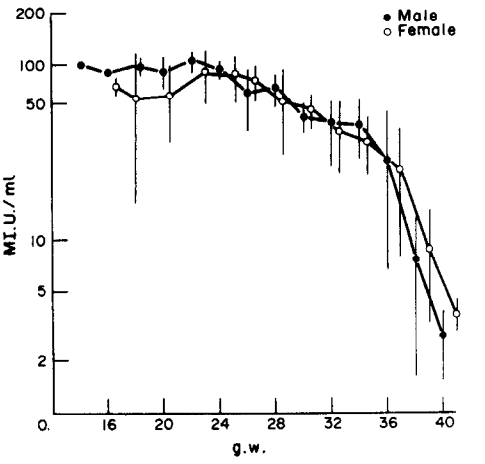

Fig. 3. Fetal plasma LH by gestational week.

female FSH levels increase to 96.0 ± 16.9 mi.U./ml roughly 20 times higher than male fetuses of the same stage. At about 30 wks plasma FSH in female fetuses decreases to 59.6 ± 24.2 mi.U./ml, after about 30 wks, the plasma FSH levels show a dramatic decrease making sexual differentiation no longer feasible after 34 weeks of fetal development.

2. *Neonatal FSH levels* (*Fig.* 2). At 24 h post partum, male neonatal FSH was 4.9 ± 1.9 mi.U./ml and female FSH was 5.5 ± 0.9 mi.U./ml. Plasma FSH levels in both male and female neonates decrease following the 24 h post partum sampling and the 6th day post partum are female: male (3.5 ± 0.5:2.4 ± 0.7 mi. U./ml), thereafter both male and female neonate plasma FSH levels show a tendency to increase. and the 10th day postpartum female: male FSH is 5.3 ± 0.9:3.8 ± 0.6 mi.U./ml respectively. These findings indicate that during the first 10 days of neonatal life, female FSH levels are consistently higher than male plasma FSH levels, although these disparities in concentration are of no statistical significance.

3. *Fetal plasma LH levels* (*Fig.* 3). At about 20 wks of fetal development, male:female LH levels are 106.0 ± 11.01, 90.4 ± 27.0 mi.U./ml respectively, and thereafter show a slight decrease. At 35–36 wks of development LH levels show a sharp decrease with no detectable significance between male and female fetuses.

4. *Neonatal plasma LH levels* (*Fig.* 4). 24 h postpartum neonatal plasma LH determinations showed that male:female levels were 5.2 ± 2.0:5.8 ± 1.0 mi.U./ml respectively, or slightly higher than levels determined in term fetuses. After the first 24 h, plasma LH levels sharply decreased in both male and female neonates and in the sixth postpartum day were 2.4 ± 0.1:3.1 ± 0.8 mi.U./ml respectively, or roughly the levels seen at term. Plasma LH levels in both male and female neonates then showed an increase. and on the tenth postpartum day was 4.2 ± 0.4:4.9 ± 0.1 mi.U./ml respectively, showing that as in the case of FSH, LH levels in the first 10 postpartum days are higher in female neonates than in male, however, as with FSH, no statistical significance could be detected.

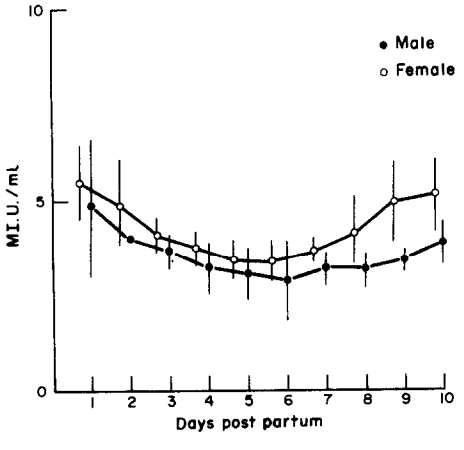

Fig. 2. Neonatal peripheral plasma FSH.

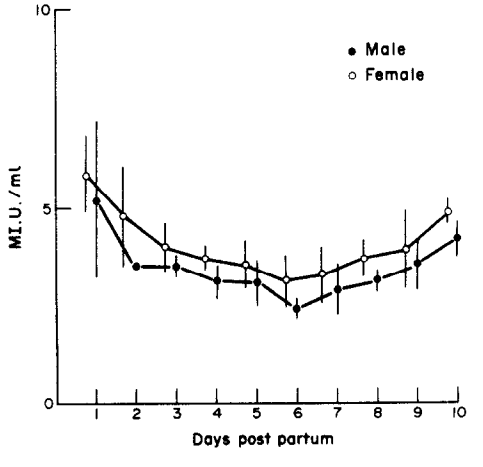

Fig. 4. Neonatal peripheral plasma LH.

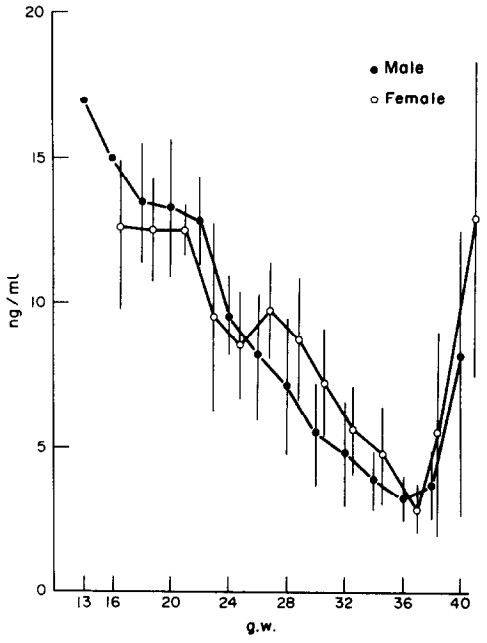

Fig. 5. Fetal plasma HCG by gestational week.

5. *Fetal HCG levels* (*Fig.* 5). Fetal plasma HCG levels at 20 wks, of development are male:female, 12.8 ± 1.5:12.5 ± 0.8 ng/ml, thereafter decreasing to minimal levels at 35–36 wks of development, followed by an increase and at term, the male:female ratio is 8.1 ± 5.4:5.4 ± 3.5 ng/ml. Our findings show two peaks in HCG levels during fetal development, one occurring during the second trimester of pregnancy, the other at term. We were unable to determine statistically significant male:female sex differences in fetal plasma HCG levels, further, neonatal determinations revealed that immediately post partum, HCG levels rapidly decrease regardless of the sex of the neonate, and 48 h determinations were less than 1 ng/ml, 72 h postpartum specimens revealed that HCG was undetectable in both male and female neonates.

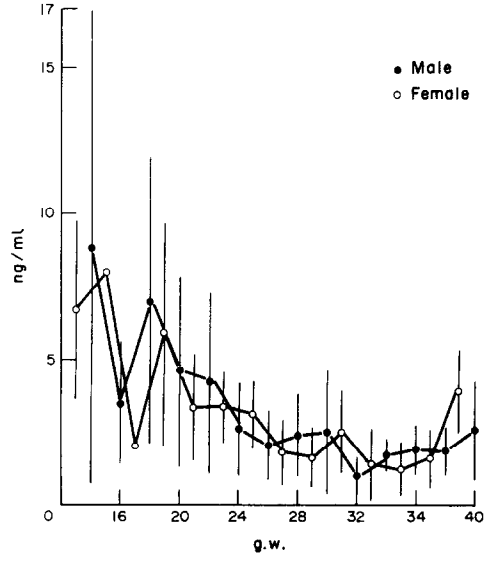

Fig. 6. Fetal plasma HCG β-subunit by gestational week.

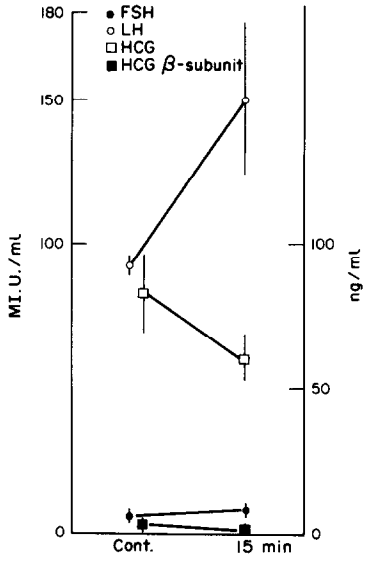

Fig. 7. Fetal plasma FSH, LH, HCG β-subunit effect of LH-RH in male.

6. *Fetal HCG-β subunit levels* (*Fig.* 6). The dynamics of the HCG-β subunit in fetal plasma correspond closely with the patterns observed with HCG and differ widely with the results obtained with LH. HCG-β subunit responds with two peaks as does HCG, one during second trimester of fetal development, the other at term. No significant sexual difference was detectable and neonates sampled at 96 h post partum revealed no detectable HCG-β subunit in the peripheral plasma.

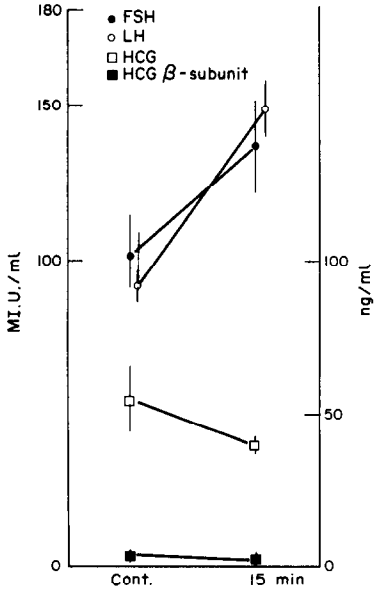

Fig. 8. Fetal plasma FSH, LH, HCG β-subunit effect of LH-RH in female.

7. *LH-RH priming.* Six fetuses (3 male:3 female) in the second trimester of fetal development were primed with LH-RH, followed by serial determinations of FSH, LH, HCG and HCG-β subunit. The prepriming controls showed male plasma FSH at 6.9 $\pm$ 1.9 mi.U./ml and female plasma FSH at 103.3 $\pm$ 12.2 mi.U./ml. At 15 min postpriming, male plasma FSH levels were 7.5 $\pm$ 1.8 mi.U./ml and female plasma FSH 137.0 $\pm$ 15.0 mi.U./ml. LH control levels were 93.3 $\pm$ 2.8 male and 91.6 $\pm$ 3.0 mi.U./ml female respectively, and at 15 min was significantly elevated to 150.0 $\pm$ 26.4 male and 148.6 $\pm$ 9.4 mi.U./ml female respectively. HCG control levels were 83.3 $\pm$ 14.0 male and 55.3 $\pm$ 10.0 ng/ml male. The 15 min levels were decreased to 60.3 $\pm$ 7.3 male and 40.0 $\pm$ 2.0 ng/ml female. HCG-β subunit control levels were 2.6 $\pm$ 0.3 male and 3.0 $\pm$ 0.9 ng/ml and 15 min levels were 2.2 $\pm$ 0.4 male and 2.3 $\pm$ 0.5 ng/ml female respectively.

The above findings seem to indicate that regardless of cross reactivity in the immunological determinations, HCG and LH have consistently differing patterns of reaction (Figs. 7 and 8), regardless of the stage of fetal development. Thus, based on the evidence of a consistently increasing LH concentration with LH-RH priming coincident with a decrease in both HCG and its β-subunit's concentrations, we feel that our method for measuring LH primarily reflects the response of fetal LH to LH-RH even in the presence of a strong cross reactivity of LH with HCG.

We then compared the results obtained by LH-RH priming of second trimester fetuses with the results obtained by priming last trimester and full term neonates.

Male FSH levels in controls and those primed with LH-RH were as follows, the 15 min post-priming controls showed a decrease to 65–72% pre priming in plasma FSH levels, whereas fetuses primed with LH-RH had 15 min levels of 102–114%. This FSH response to LH-RH was consistent in both second trimester fetuses and last trimester neonates (Table 6). In comparison, female plasma FSH in the controls decreased to 60–83% pre-priming levels at 15 min and the LH-RH primed females showed 15 min levels of 125–140% in second trimester fetuses. Last trimester female neonates showed a decrease. to 83% at 15 min in the controls, whereas the LH-RH primed neonates 15 min levels were 106–109% (Table 7). These findings indicate that in female fetuses in the second trimester of development, the response to LH-RH priming is significantly better than the response elicited in female last trimester neonates ($P = 0.01$). Fetuses in the second trimester of development showed a significant male: female response difference ($P = 0.01$), but full term neonates showed no significant sexual difference in response to LH-RH priming (Table 8).

Male LH levels in second trimester fetuses treated as controls decreased to 52–56% pre-priming levels whereas 15 min levels in male fetuses primed with LH-RH, showed an increase to 123–188% pre-priming levels. Last trimester male neonates used as controls showed a decrease in LH-levels to 66–70% that of pre-priming levels and the LH-RH primed male neonated had 15 min plasma LH levels of 112–127% that of the male neonates prior to priming (Table 9). All subjects primed with LH-RH, regardless of the stage of development showed a positive response with mid trimester male fetuses showing a better response than last trimester male neonates ($P = 0.01$).

Female plasma LH levels in second trimester fetuses used as controls decreased to 51–72% pre-priming levels, those primed with LH-RH showed

Table 6. Plasma FSH effects of LH-RH on male fetuses

		Gestational weeks	Pretreatment mi.U/ml	15′ After Administration	
				mi.U/ml	%
Mid trimester	LH-RH	16	5	5.5	110
		22	7	8	114
		22	7.8	8.2	105
		23	5.5	6	109
		24	7.5	8	106
		25	6.5	7.2	110
		26	8.8	9.0	102
		27	8.4	9.0	107
	Control	22	9.8	6.8	69
		24	7.0	4.5	65
Last trimester	LH-RH	33	4.5	4.8	106
		34	4.2	4.6	109
		35	2.5	2.6	104
	Control	34	6.8	4.9	72

* Control = Physiological saline

Table 7. Plasma FSH effects of LH-RH on female fetuses

		Gestational week	Pretreatment mi.U/ml	15′ After administration	
				mi.U/ml	%
Mid trimester	LH-RH	15	80	112	140
		18	85	115	135
		20	90	122	135
		24	92	128	140
		24	104	132	126
		25	106	134	126
		25	114	158	138
		26	118	148	125
		26	125	164	131
		27	95	130	136
	Control	16	75	45	60
		19	95	62	68
		24	98	82	83
		26	100	75	75
Last trimester	LH-RH	33	7.5	8.2	109
		35	8	8.5	106
	Control	34	6	5	83

* Control = Physiological saline

15 min LH levels of 146–173% greater than those prior to priming. Female last trimester neonates used as controls showed a decrease in plasma LH levels to 57%, and LH-RH primed neonates showed 15 min levels of 116–120%, indicating that as with male fetuses and neonates, female fetal LH response to LH-RH was better than that of female last trimester and term neonates ($P = 0.01$) (Tables 10, 11).

II. Fetal testicular androgen synthesis

1. *Fetal and neonatal plasma testosterone levels in pregnancy and at parturition* (*Fig.* 9). Maternal peripheral plasma testosterone levels increase from early pregnancy to nearly double the early pregnancy levels at term. In comparison to maternal plasma testosterone levels, the fetal male and female plasma testosterone were as follows. Male plasma testosterone levels at 16 wks of fetal development were 0.99 ± 0.32 ng/ml, at 20 wks increased to 1.02 ± 0.28 ng/ml. After 20 wks of fetal development, testosterone levels gradually decreased and at term, was roughly 1/2 the levels observed at 16–20 wks. Female fetal plasma testosterone levels at 16 wks was 0.36 ± 0.09 ng/ml, but thereafter until 24 wks of fetal development, female testosterone levels gradually increase. 16–20 wks of fetal development, the male:female plasma testosterone levels are roughly 3:1, showing a clearly significant sexual difference between male and female fetuses in the second trimester of preg-

Table 8. Effects of LH-RH on neonatal plasma FSH

		Hours post partum	Pretreatment mi.U./ml	15′ After administration	
				mi.U./ml	%
♂	LH-RH	23	3.0	4.5	150
		72	2.4	2.6	108
		92	2.1	2.5	119
	Control	28	2.8	2.2	78
♀	LH-RH	19	3.6	3.8	105
		33	4.7	5.2	109
		44	4.0	4.1	102
		90	4.5	5.5	122
	Control	23	4.2	3.5	82

* Control = physiological saline

Table 9. Plasma LH effects of LH-RH on male fetuses

		Gestational week	Pretreatment mi.U./ml	15′ After administration mi.U./ml	%
Mid trimester	LH-RH	16	95	160	168
		22	90	170	188
		22	120	180	150
		23	70	110	157
		24	105	130	123
		25	75	118	157
		26	95	120	126
		26	80	115	126
		27	65	85	130
	Control	22	85	48	56
		24	100	52	52
Last trimester	LH-RH	33	55	70	127
		34	38	48	126
		35	25	28	112
	Control	30	80	56	70
		36	18	12	66

* Control = physiological saline

nancy. This sexual difference seen in second trimester fetuses is no longer detectable at term in reference to fetal plasma testosterone levels.

2. *Neonatal and nursing infant plasma testosterone levels* (*Fig.* 10). Plasma testosterone levels were determined serially from immediately post-partum to the 7th day post-partum. Male neonates showed on unstable range of 0.21–1.12 ng/ml during this period and no definite pattern was discernible. Female neonates immediately post-partum had plasma testosterone levels of 0.77 ± 0.22 ng/ml. However, in 4 female neonates 1 day post-partum, only one reading of 0.23 ng/ml was obtainable. No female neonatal plasma testosterone was detectable thereafter through the 7 days of sampling. In nursing infants, male infant plasma testosterone levels were consistently detectable, whereas females of the same stage of development plasma testosterone was consistently undetectable.

3. *Fetal and immediately post-partum neonatal*

Table 10. Plasma LH effect of LH-RH on female fetuses

		Gestational week	15′ After Pretreatment mi.U./ml	Administration mi.U./ml	%
Mid trimester	LH-RH	15	30	50	166
		18	100	152	152
		20	90	138	153
		24	85	132	155
		25	95	152	160
		25	90	156	173
		26	70	115	164
		26	92	135	146
		27	94	150	159
	Control	16	75	38	51
		19	45	24	53
		24	60	42	70
		26	80	58	72
Last trimester	LH-RH	33	40	48	120
		35	50	58	116
		36	25	30	120
	Control	36	35	20	57

* Control = physiological saline

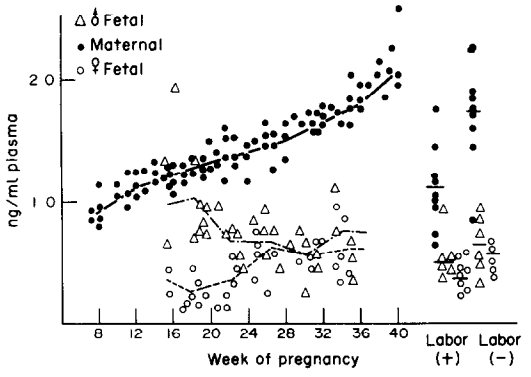

Fig. 9. Materno–fetal plasma testosterone levels in pregnancy and at parturition.

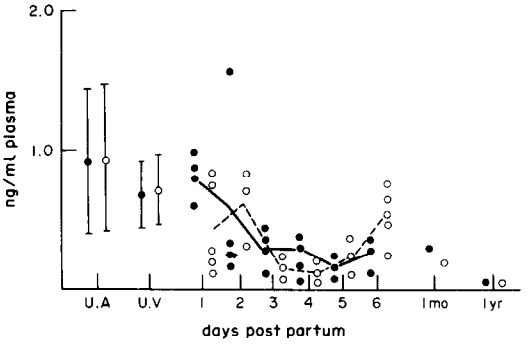

Fig. 10. Neonate and nursing infant plasma testosterone levels.

plasma 4-ene-androstenedione levels (*Fig.* 11). Maternal peripheral plasma 4-ene-androstenedione levels in pregnancy were 1.06–2.69 ng/ml, with a tendency toward a slight increase near term. Fetal and neonatal plasma 4-ene-androstenedione were 0.44–1.80 ng/ml irrespective of sex, and we were unable to determine a consistent pattern.

4. *Neonatal and nursing infant plasma 4-ene-androstenedione levels* (*Fig.* 12). As in fetuses and immediately postpartum neonates the plasma 4-ene-androstenedione levels in neonates and nursing infants showed no significant differences between males and females.

5. *Materno-fetal plasma 5α-DHT levels in pregnancy and at the time of full term delivery* (*Tab.* 12) Materno-fetal plasma 5α-DHT levels in pregnancy and at parturition were undetectable in many cases making a definite pattern or sex differences unattainable.

6. *Plasma 5α-DHT levels in neonates* (*Tab.* 13). Neo-

Table 11. Effects of LH-RH on neonatal plasma LH

		Hours post partum	Pretreatment mi.U./ml	15′ After administration	
				mi.U./ml	%
♂	LH-RH	23	3.0	4.1	136
		72	3.3	4.6	140
		92	2.7	3.1	115
	Control	38	3.8	2.4	63
♂	LH-RH	19	4.7	5.0	106
		33	6.0	9.0	150
		44	4.8	8.1	168
	Control	46	4.3	3.8	90

* Control = physiological saline

Table 12. Materno-fetal plasma 5α-dihydrotestosterone levels to pregnancy and at parturition ng/ml

	12 week	20		28		36		term deliveries			
Maternal	0.0176	0.0176		0.0262		0.0283		0.1309			
	0.0336	0.0199		non		0.0304		0.1308			
	0.0598	non		non		0.0157		0.1746			
	non	non		non		non		0.0800			
	non	non		non		non		non			
	non	non		non		non		non			
		♂	♀	♂	♀	♂	♀	U.A.		U.V.	
Fetal		0.0331	0.0568	0.1023	0.0738	0.1000	0.0563	♂	♀	♂	♀
		0.1785	0.0729	0.1428	0.1693	0.0444	non				
		0.0500	0.624	0.1500	non	0.1422		0.1309	0.0952	0.0937	0.0690
			non	0.1770				0.1116	0.1256	0.0888	0.0596
			non	0.1047				non	0.1285	non	non
								non	non	non	non

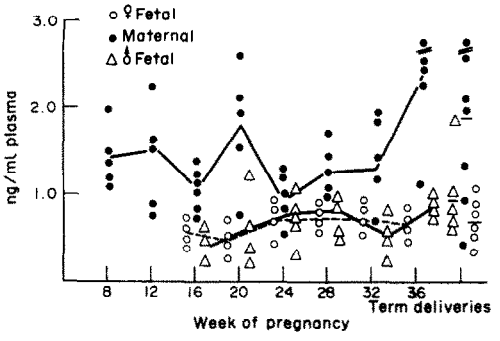

Fig. 11. Materno–fetal plasma 4-ene-androstenedion levels in pregnancy and at parturition.

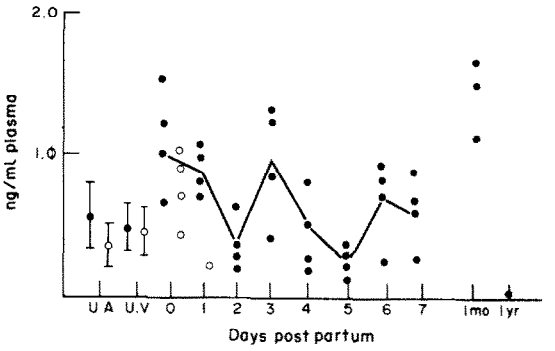

Fig. 12. Neonate and nursing infant plasma 4-ene-androstenedion levels.

natal plasma 5α-DHT levels as in fetuses were undetectable in many cases allowing no definite conclusions.

III. Testicular LH and HCG receptors as seen using fluorescent antibodies (Fig. 13) Interestitial cells show fluorescence but not the seminiferous tubules.

DISCUSSION

The fetal testicles are said to be involved in endocrine activity and the control of the development of the male fetal genital system. This can be substantiated by the appearance of male:female sexual differences in fetal androgen levels at from 10–16 wks. of pregnancy, the period at which genital tract differentiation is said to occur.

Androgen metabolism in pregnancy has not been as thoroughly investigated as the estrogens, however, it has been established that androgen synthesis occurs in the maternal organs, in the placenta and in the fetal organs.

We determined fetal androgens from 16 wks of pregnancy (fetal age 14 wks) and found that fetal plasma 4-ene-androstenedione and testosterone were in lower concentrations than in the maternal peripheral plasma. Testosterone showed definite significant sexual differences up to about 24 wks of pregnancy, but no male:female sex differences were observed in 4-ene-androstenedione levels.

The fetal testicular metabolic pathways for the synthesis of testosterone has been determined *in vitro* by Bloch[8] and Rice[9], and testosterone being detected in fetal plasma seems to indicate fetal testosterone synthesis. In mature males, androgen, more specifically, testosterone synthesis is known to occur in the Leydig cells. Testicles of fetuses of 4 months development show histologically a broad interstitial layer containing an abundance of Leydig cells, and under electron microscopy show the specific properties attributable to steroidogenetic cells: An abundance of microsomes, larger mitochondria containing numerous cristae and the development of the ovaries.

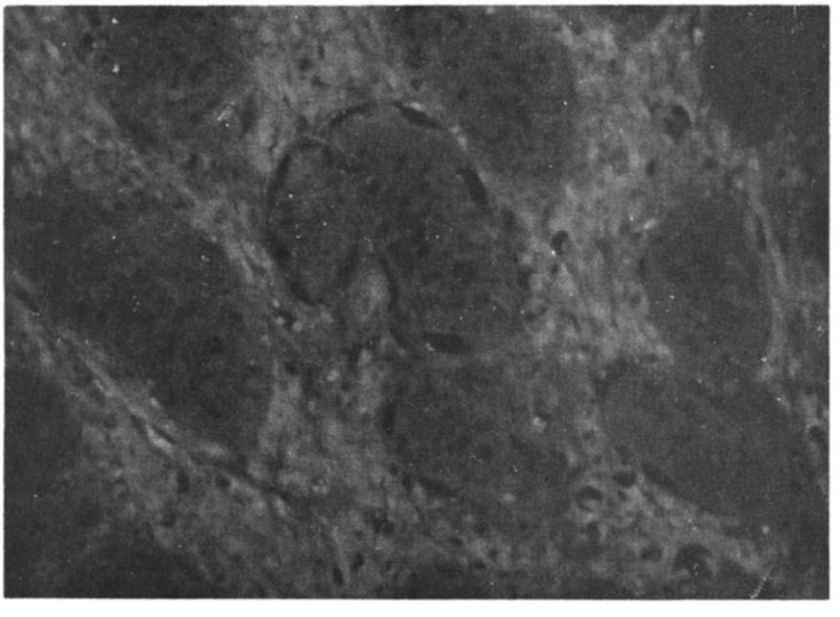

Fig. 13.

Table 13. 5α-Dihydrotestosterone levels of human newborn ng/ml

1 day		2		3	
♂	♀	♂	♀	♂	♀
0.140	0.064	0.041	0.080	0.181	0.067
0.128	0.136	0.169	0.125	0.185	non
	0.077	0.100	non	0.154	non
	0.090	non	non	0.176	non
	non				
4		5		6	
♂	♀	♂	♀	♂	♀
0.146	0.024	0.084	0.111	0.047	0.110
0.111	non	non	non	non	0.177
non	non	non	non	non	0.058
					non

golgi apparatus observed all tend to indicate an increased activity in these cells. Further, the detection of 17β-hydroxylase and 3β-hydroxysteroid dehydrogenase activity seem to also suggest active steroidogenesis relatively early in fetal development.

Jost[1] and Reyes[5] report that human fetal testicles have the ability to synthesize testosterone from early in fetal development, and the fetal plasma testosterone peak coincides with the period at which the Wolffian duct differentiates. Soon after the maternal peripheral plasma HCG levels decrease, an involution of the cellular elements of the fetal testicular interstitia occurs, accompanied by a decrease in plasma testosterone. Although, the effects of HCG and LH on androgen synthesis and their involvement in testicular maturation are not as yet well understood, Eiknes and Osawa have reported that HCG and LH administered intravenously cause a rapid increase in plasma testosterone levels. Kawaoi[6] utilizing fluorescent antibodies in rat testicles, identified the LH receptors in the interstitia. Our fluorescent antibody series on human fetal testicles identified both LH and HCG receptors in the testicular interstitia. From these findings it may be speculated that LH and HCG stimulate testicular synthesis of testosterone resulting in elevated fetal plasma testosterone levels. Sakamoto *et al.*[7] have reported that early in fetal development, testicular androgen synthesis is stimulated by HCG and in late pregnancy by hypophyseal gonadotropins. These findings may lead to the assumption that there may be sex difference in HCG or LH. However, as we have previously mentioned, our findings are that regardless of the stage of pregnancy, fetal plasma LH and HCG show no male:female sex differences.

However, the testes develop in anencephalic monsters and fetuse with hypophyseal atresia (Ch'in[10], Bearn[11]). We were able to confirm Ross[12] in establishing that the HCG-β subunit is transmitted to the fetuses, and since the testicle differentiates and synthesizes testosterone prior to the stage of development during which the hypophysis attains function, it is hard to disprove the assumption that early in fetal development, HCG has an LH like function. However, neonatal plasma testosterone levels and hypophyseal gonadotropin levels show male:female sex differences and Laron[13] has reported that frequently, anencephalic monsters have external genital defects and undescended testicles suggesting that prior to definitive sexual differentiation, fetal LH and FSH may be actively engaged in the process of sexual differentiation.

4-Ene-androstenedione which is a metabolic precursor of testosterone shows no detectable male: female sex differences in fetal plasma, leaving its biological significance uncertain.

5α-DHT a 5α-reduction metabolite of testosterone is stored in adult cells, especially the nuclei, and is recognized as a true androgenic hormone. Our investigations revealed that in fetuses, 5α-DHT is often undetectable, however, Schultz *et al.* reported that 5α-DHT is essential in male fetuses to induce development of the urogenital sinus and genital tubercle. We feel that the relationship between testosterone and 5α-DHT and their effects on fetal development with regards to receptor or target is as yet incompletely understood and require further intensive investigations.

Male fetal plasma FSH levels 15–32 wks of development was lower than that of females of the same period of development, and our results were as with those of Grumbach[2, 3] and Reyes[5], and further our results on fetal pituitary FSH were in close agreement with Grumbach[3]. The male:female sex differences in FSH levels is said to be due to the negative feedback of testosterone, as Grumbach *et al.*[2, 3] have reported. Fetal testosterone is first synthesized at about 10 wks of fetal development, peaking at 17–20 wks and decreases after about 22 wks. In contrast, as FSH levels throughout this period remained relatively stable, it is difficult to explain the relationship of testosterone to FSH in fetal plasma with a feed-back mechanism only, because of the female FSH pattern as well as the male:female LH dynamics and their effects on the hypothalamo-hypophyseogonadal system's functional automaticity, its development of feed back mechanisms, and or the involvement of the fetoplacental unit in the synthesis of steroids, may all have either direct or indirect influence on the endocrine pattern observed.

Female fetuses ovaries develop primordial oocytes (oogonia) at about 5 1/2 wks of fetal development and these cells are said to be abundant in the ovarian cortex (Sawasaki[14]). Falin[15] reported that the primordial follicles become most numerous at 22–24 wks of fetal development and that only a portion of them become primary follicles. Ross[16] reported that at 6–7 months of fetal development, the primary follicles develop into Graafian follicles. Ch'in[10] reported that in female anencephalic monsters there are abnormalities in the primordial follicles, indicating that hypophyseal gonadotropins especially FSH are indispensable for the differentiation and maturation of the

Our investigations indicated that the release of FSH in female fetuses peaks at 22–28 wks leaving the possibility of fetal FSH being involved in ovarian maturation.

The human fetal hypothalamus remains largely uninvestigated, thus the hypothalamo-hypophyseo-gonadal system in fetus remains as yet for the most part a mystery. Levina[17] using hypothalamic extracts cultured human fetal pituitaries and reported that male pituitary cultures began to elaborate FSH and LH at the fetal age of 24 wks whereas female cultures began to elaborate FSH and LH at 19 wks.

Oka[18] reported that fetal pituitary cultures begin to be sensitive to LH-RH stimulation at a fetal age of 10 wks and are capable of elaborating LH. Our results indicate priming fetuses and neonates with LH-RH stimulates the release of LH and FSH. Although LH levels showed no male:female sex differences, FSH levels show significant female:male reactions beginning in the second trimester of fetal development. Further, it is not yet clear whether LH-RH's effect on the anterior hypophysis is to merely release of LH and FSH, or to stimulate both synthesize and release LH and FSH. In general, LH-RH is said to increase plasma FSH and LH levels and decrease anterior hypophyseal FSH and LH levels. Samli[19] using [^{14}C]-leucine and [^{14}C]-glucosamine inhibited with puromycin to prevent their being bound to LH, observed the effects of LH-RH. He found that LH-RH stimulates the release, but not the synthesis of FSH and LH. Wakabayashi[20] reported that the effect of LH-RH is difficult to impede with either puromycin or actinomycin D. Further, as we were unable to detect female:male differences in fetal LH levels nor could we detect any differences in response to LH-RH, it is difficult to postulate a female:male sexual difference in LH-RH release.

Since the fetal plasma FSH dynamics during the second trimester of pregnancy show definite female:male differences, it becomes difficult to explain the LH-RH level differences during the critical period of differentiation based on a simple feed back mechanism of testosterone, thus the presence of the FSH sex difference as well as its significance require further investigation.

The decrease in fetal FSH, LH levels near term is assumed to be due to either the increase in circulating steroids, or perhaps as Grumback claims, a feed back mechanism in the hypothalamo-hypophyseo-gonadal system, which has attained automaticity. Regardless of the mechanism involved, neonates, both male and female show a tendency toward a decrease in FSH, LH levels in the immediately post partum period, followed by a tendency for both FSH and LH levels to increase a few days post partum. Further, Charters[21] reported that stress is capable of causing decrease in FSH and LH levels, making the stresses associated with the changing endocrine milieu encountered near term, together with the physical stresses imposed by the birth processes have an influencing effect. Thus, it is difficult to negate the assumption that relatively early in fetal development, there developes an interdependence of the hypothalamo-hypophyseo-gonadal system.

CONCLUSIONS

The peripheral plasma gonadotropins in fetuses and neonates plus fetal testicular androgens were serially followed by RIA, and a concurrent investigation was performed on the effects of LH-RH priming.

Second trimester fetuses show a definite sexual difference in FSH levels, but LH levels do not. The dynamics of hypophyseal gonadotropins in fetuses may be due to fetal testicular testosterone plus the other sex steroids affecting the in utero fetal hypothalamo-hypophyseo-gonadal system which has attained automaticity, by a feed back mechanism.

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