GONADOTROPHINS DURING SECOND TRIMESTER OF PREGNANCY:
I. LH AND hCG LEVELS IN MATERNAL SERUM AND AMNIOTIC FLUID AND THEIR RELATIONSHIP TO THE SEX OF THE FOETUS

By
B. Dattatreyamurty, A. R. Sheth, T. V. Purandare, R. Companiwalla and U. Krishna

ABSTRACT
Luteinizing hormone and chorionic gonadotrophin levels were selectively measured by using radioimmunoassays in 98 maternal sera and 116 amniotic fluid samples obtained during 10–20 weeks of pregnancy. Levels of hCG in serum were clearly high during 10–14 weeks and thereafter declined gradually. In contrast, serum concentrations of LH during 10–20 weeks were either unmeasurable (< 1 ng/ml) or lower than those observed during the luteal phase of the menstrual cycle suggesting a decreased responsiveness of pituitary and/or a higher clearance rate for LH during this period of pregnancy. Neither LH nor hCG levels in maternal sera showed significant differences between male and female foetus bearers. A striking similarity was observed between maternal serum and amniotic fluid hCG patterns, despite hCG levels in maternal sera being always higher (1.5–26.9 fold). On the other hand amniotic fluid concentrations of LH became elevated following 12 weeks of gestation while maternal serum LH continued to be at low levels until 20 weeks. Furthermore a sexual dichotomy was observed in amniotic fluid LH concentrations but not in

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hCG levels during 14–20 weeks of pregnancy, with significantly lower LH levels in male foetus bearers than in female foetus bearers. Of interest is the clear demarcation in LH levels at 16 weeks of gestation. This sequential pattern of change in the concentrations of amniotic fluid LH is similar to those patterns reported by other investigators for foetal serum and pituitary LH during 10–20 weeks of gestation suggesting that the foetus may be the source of the increased levels of LH in amniotic fluid following 12 weeks of pregnancy.

The physiological function of the pituitary-ovarian axis and its interrelationships with placental endocrine activities during pregnancy are incompletely understood. Previous studies on the assessment of pituitary gonadotrophic function during pregnancy by monitoring circulatory FSH have shown conflicting results. Thus serum FSH levels during pregnancy have been reported to be either undetectable, low (Jaffe et al. 1969; Parlow et al. 1970) or high (Faiman et al. 1968; Hanson et al. 1970). Detailed data on LH levels during pregnancy have not been readily available because of its cross-reactivity with hCG.

While the present study was under progress, Clements et al. (1976) reported a study in which the development of a homologous β-hLH radioimmunoassay allowed the investigators to monitor selectively amniotic fluid concentrations of LH in male and female foetus bearers during mid-pregnancy. However, similar studies on maternal serum LH have not yet been reported. In the present study, we employed a different type of radioimmunoassay (RIA) heterologous LH-RIA (Dattatreyamurty et al. 1976) to measure selectively the levels of LH in maternal serum and amniotic fluid during 10–20 weeks of pregnancy. Further, the data were evaluated to examine whether there are differences in serum and amniotic fluid LH levels between male and female foetus bearers. hCG levels in these biological fluids were taken for comparison.

MATERIALS AND METHODS

Ten to 15 ml of amniotic fluid was obtained transabdominally between 10–20 weeks of gestation, from women undergoing medical termination of pregnancy (MTP). The amniotic fluid samples were centrifuged at 1000 r.p.m. for 10 min. The cell pellet (from samples collected during 14–20 weeks of pregnancy) was utilized for prenatal sex determination by X and Y chromatin studies. The supernatant was used in the present study for hormonal estimation. Blood samples were also obtained from some of these women. Samples were allowed to clot and the sera separated by centrifugation at 4°C. Both amniotic fluid and serum samples were stored at −20°C until they were analysed for hormonal levels.

Purified hCG (CR 119) and hLH (LER 960) were radioiodinated with 125I to a specific activity of 90–145 µCi/µg using choramine-T method (Greenwood et al. 1963) as modified by Midgley (1966).
Specific hCG levels in the maternal sera and amniotic fluid samples were determined by a radioimmunoassay using an antiserum to β-hCG (SB 6) and radiiodinated hCG. This radioimmunoassay was carried out essentially as reported previously (Dattatreya-anurty et al. 1975). The sensitivity of the assay was 0.25–0.5 mIU per assay tube. The intra- and inter-assay coefficient of variations for samples were less than 9 per cent. hCG levels in samples are expressed in terms of 1U/ml using the 2nd IR-hCG as standard.

A specific heterologous radioimmunoassay “[125I]hLH + anti-oLH serum” which was reported previously (Dattatreya-anurty et al. 1976) was employed in the present study to selectively measure LH levels in maternal sera and amniotic fluid samples. The sensitivity of the assay was 0.3–0.5 ng of LER 960 per assay tube. Serial dilutions of amniotic fluid sample and hLH (LER 960) gave parallel dose-response lines. The intra- and inter-assay coefficient of variations for samples were less than 11 per cent. In the LH radioimmunoassay, purified hCG in amounts up to 1000 ng (4.9 IU) per assay tube, a value which corresponds to approximately 25 IU per ml of pregnancy serum, could not effectively inhibit the binding of radiiodinated hLH by the antibody. Subunits of hCG were as ineffective as intact hCG in this radioimmunoassay. LH levels in samples are expressed in terms of ng of LER 960 per ml of sample. (34 ng LER 907 is equivalent to 1 ng LER 960).

Statistical evaluation of the data on hormonal levels was carried out using Student’s t-test.

Fig. 1.
Serum hCG concentrations in women bearing male foetus (●) or female foetus (▲) are expressed in IU of the 2nd IR-hCG/ml on the ordinate against gestational age in weeks on the abscissa. Mean concentration of serum hCG in male foetus bearers ————, and in female foetus bearers ————.
RESULTS

A total of 98 maternal sera and of 116 amniotic fluid samples obtained during 10–20 weeks of pregnancy were analysed for hCG and LH levels. Information about the foetal sex was available only for samples obtained during 16–20 weeks of gestation and accordingly the data obtained from the analysis of these samples were used to examine the differences in LH and hCG levels between male and female foetus bearers.

Chorionic gonadotrophin

Serum hCG concentrations in women during 10–14 weeks of gestation were in the range 6.2–22.3 IU/ml with a mean value of 14.21 ± 0.81 (n = 31). Circulating hCG concentrations in women during 16–20 weeks of pregnancy are shown in Fig. 1. Following 14 weeks of gestation, serum hCG levels declined gradually. No significant differences were observed in maternal serum hCG concentrations between male and female foetus bearers during 16–20 weeks of pregnancy (Table 1).

Amniotic fluid samples obtained from 10–14 weeks of pregnancy had maximum hCG concentrations with a mean value of 6.82 ± 1.8 IU/ml (n = 15). Fig. 2 illustrates the amniotic fluid hCG levels in women during 14–20 weeks of pregnancy. Following 14 weeks of pregnancy, amniotic fluid hCG levels declined in a way similar to those in maternal circulation. No significant differences were observed in amniotic fluid hCG levels between male and female foetus bearers during 16–20 weeks of gestation (Table 1).

Comparison of maternal serum and amniotic fluid levels of hCG

In the 21 matched samples obtained from women during 12–20 weeks of gestation, serum hCG levels were generally higher than those of amniotic fluid samples (Fig. 3). The ratio of serum to amniotic fluid hCG varied between 1.56–26.91 with a mean value 9.83. A significant correlation (r = 0.6594, P < 0.001) was observed between serum and amniotic fluid hCG levels in matched specimens.

Luteinizing hormone

Fig. 4 shows the dose-response lines of intact hLH and β-subunit of hLH in "[125I]hLH + anti oLH serum" radioimmunoassay. The immunoreactive patterns of intact hLH and β-hLH were different as they displayed different slopes and affinities. The slopes calculated from probit regression lines for intact hLH and β-hLH were respectively -1.13 and -0.393. This discrimination became more obvious as the concentration of β-hLH increased since the di-
Table 1.

hCG levels* in maternal serum and amniotic fluid.

<table>
<thead>
<tr>
<th>Sex of the foetus</th>
<th>Gestation</th>
<th>16 weeks</th>
<th>18 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Maternal serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.23 ± 0.62</td>
<td>9.43 ± 0.55</td>
<td>7.49 ± 0.5</td>
<td>8.24 ± 0.57</td>
</tr>
<tr>
<td>(11)</td>
<td>(10)</td>
<td></td>
<td>(12)</td>
<td>(10)</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.22 ± 1.04</td>
<td>4.16 ± 0.81</td>
<td>3.86 ± 0.89</td>
<td>3.01 ± 0.64</td>
</tr>
<tr>
<td>(23)</td>
<td>(20)</td>
<td></td>
<td>(12)</td>
<td>(10)</td>
</tr>
</tbody>
</table>

* Expressed as mean ± se (n) in IU of the 2nd IR-hCG per ml.

Levels show no statistically significant difference between sexes of foetuses.
Amniotic fluid hCG concentrations in women bearing male foetus (●) or female foetus (▲) are expressed in IU of the 2nd IR-hCG/ml on the ordinate against gestational age in weeks on the abscissa. Mean concentration of amniotic fluid hCG in female foetus bearers ---, and in male foetus bearers ----.

Fig. 2.

Comparison of maternal serum and amniotic fluid (AF) hCG levels in paired samples. Serum and AF levels of hCG are expressed in IU/ml.

Fig. 3.
Fig. 4.
Dose-response lines for intact hLH-LER 960 (●) and β-hLH (▲) in the heterologous radioimmunoassay using [125I]hLH and anti-oLH serum absorbed with hCG (Dattatreyamurty et al. 1976).

Fig. 5.
Serum LH concentration in women bearing male foetus (●) or female foetus (▲) are expressed in ng of LER 960/ml on the ordinate against gestational age in weeks on the abscissa. Mean concentration of serum LH in male foetus bearers ————, and in female foetus bearers ————.
Table 2.
LH levels* in maternal serum and amniotic fluid.

<table>
<thead>
<tr>
<th>Gestation</th>
<th>16 weeks</th>
<th>18 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex of the foetus</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Maternal serum</td>
<td>3.09 ± 0.35 (11)</td>
<td>2.87 ± 0.28 (10)</td>
<td>2.89 ± 0.35 (11)</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>11.56 ± 0.69a (20)</td>
<td>18.96 ± 0.93a (20)</td>
<td>15.30 ± 1.46b (11)</td>
</tr>
</tbody>
</table>

* Expressed as mean ± se (n) in ng of purified LH (LER 960)/ml (1 ng = 34 ng LER 907 reference standard).

Significant levels between sexes of foetuses
a $P < 0.001$

b $0.05 < P < 0.1$

c $0.001 < P < 0.01$

LH levels during the *luteal phase of the normal menstrual cycle* (n = 5) = 5.96 ± 0.17 ng/ml (range, 5.03–8.2; n = 23).
rection of the dose-response line changed and it remained at a plateau when 100 ng or more of $\beta$-hLH were added per assay tube. Furthermore, the relative affinities of intact hLH and $\beta$-hLH in the radioimmunoassay were quite different. $\beta$-subunit of hLH required 19.5 times more mass relative to intact hLH for 54 per cent inhibition (the nearest to 50 per cent) in the radioimmunoassay.

Fig. 5 shows serum LH levels in women during 16–20 weeks of pregnancy, as determined by the above heterologous LH radioimmunoassay. LH concentrations during 16–20 weeks of pregnancy were either undetectable (<1 ng/ml) or lower than those observed during the luteal phase of the menstrual cycle (Table 2, $P < 0.001$). Maternal serum LH concentrations during 10–14 weeks of pregnancy were also low or undetectable with a mean value of 2.93 ± 0.29 (n = 31). No significant differences were observed in serum LH levels between male and female foetus bearers during 16–20 weeks of gestation (Table 2).

A few amniotic fluid samples (n = 4) obtained at 10 weeks of pregnancy had LH concentrations undetectable to less than 3 ng/ml. Amniotic fluid LH levels became elevated with a mean value of 6.03 ± 0.94 ng/ml (n = 6) at 12 weeks of pregnancy. Between 14–20 weeks of gestation, LH concentrations increased in both male and female foetus bearers (Fig. 6). Furthermore during

**Fig. 6.**
Amniotic fluid LH concentrations in women bearing male foetus (●) or female foetus (▲) are expressed in ng of LER 960/ml on the ordinate against gestational age in weeks on the abscissa. Mean concentration of serum LH in male foetus bearers ———, and in female foetus bearers ————.
this period of pregnancy, the amniotic fluid LH levels were significantly higher in female foetus bearers than in male foetus bearers \( (P < 0.001) \) (Table 2). Of interest was the clear demarcation in the LH concentration between male and female foetus bearers at 16 weeks of gestation (Fig. 6).

**DISCUSSION**

Amniotic fluid and maternal serum hCG levels in the present study and placental hCG concentrations in a previous study of Wide & Hobson (1974) showed no significant differences between male and female foetus bearers during the 2nd trimester of pregnancy. This is contrary to the situation at the end of pregnancy (Danielsson 1965; Crosignani et al. 1972; Hobson & Wide 1974). Although there is a striking similarity in amniotic fluid and maternal serum hCG patterns, hCG levels in maternal sera are always higher. Concentrations as high as 1.5–26.9-fold have been observed for maternal sera in the present study. It must be noted that both hCG and LH are immunologically and biologically related and obviously specific assays are required for selective measurement of LH in body fluids during pregnancy.

The use of a heterologous LH radioimmunoassay in the present study and of a homologous \( \beta \)-hLH RIA in recent studies of Clements et al. (1976) and Kaplan & Grumbach (1976) permitted the measurement of LH in biological fluids during pregnancy. Although the two assay systems are comparable in their high degree of specificity, they can be distinguished on the basis of the immunoreactivity patterns of intact hLH and \( \beta \)-subunit of hLH. In the homologous \( \beta \)-hLH assay, \( \beta \)-hLH has been shown to display a higher affinity to antibody than intact hLH (Clements et al. 1976). On the other hand, in the present study, intact hLH displayed a dose-response line with a higher affinity and slope than \( \beta \)-hLH. In other words, the present assay when employed for measuring LH in body fluids, would preferentially measure intact hormone (regular) if hormone subunits are present in addition to regular hormone in body fluids as shown by other investigators (Ryan 1969; La Burthe et al. 1973; Prentice & Ryan 1975). This is supported by our earlier observation that this assay gives hormonal estimates in close agreement with those derived by a conventional bioassay (Dattatreyamurty et al. 1976).

During 10–20 weeks of pregnancy, LH and hCG patterns in amniotic fluid appeared to be different. LH levels in amniotic fluid were low (\(< 3 \text{ ng/ml}\)) at 10 weeks and became elevated between 12–20 weeks of pregnancy, while the hCG concentrations were showing a gradual decline. As has been noted in the study of Clements et al. (1976), we also observed a sexual dichotomy in the amniotic fluid LH concentrations during 14–20 weeks, with significantly lower levels in male foetus bearers than in female foetus bearers.
To our knowledge, there have been no reports on maternal serum LH in male and female foetus bearers. In the present study, we noticed a striking difference between amniotic fluid and maternal serum LH patterns during 10–20 weeks of pregnancy. Thus maternal serum LH levels were consistently low throughout this period, while the levels in amniotic fluid were elevated between 12–20 weeks of pregnancy. Further more our studies failed to reveal a sexual dichotomy in maternal serum LH levels during 16–20 weeks of pregnancy. The observation that maternal serum LH levels during 10–20 weeks of pregnancy were lower than those observed during the luteal phase of the menstrual cycle (Table 2), possibly suggests a decreased responsiveness of pituitary and/or a higher clearance rate for LH during 10–20 weeks of pregnancy. It is of interest to note that Jaffe et al. (1969) and Parlow et al. (1970) have shown low circulating FSH levels during pregnancy. However other investigators (Faiman et al. 1968; Hanson et al. 1970) have found the FSH levels to be high. In a later report, Jeppsson et al. (1974) confirmed the FSH levels during pregnancy to be low and further they suggested that the low levels are due to decreased pituitary responsiveness to LH-RH during pregnancy.

The fact that the LH concentrations in amniotic fluid increase following 12 weeks of gestation, while they remain low in maternal circulation, suggests that a source other than maternal circulation may be responsible for the increased hormonal concentrations in amniotic fluid. Previous in vitro studies (Levina 1970; Groom et al. 1971; Oka 1975) have demonstrated that the foetal pituitary gland in organ culture has the capacity to secrete LH and FSH. The concentrations of LH in foetal pituitary (Kaplan & Grumbach 1976) and circulation (Clements et al. 1976; Kaplan & Grumbach 1976) have been shown to increase from 12 weeks of gestation, a pattern which is similar to that observed for amniotic fluid in the present study. It is likely that the increased LH levels in amniotic fluid during 12–20 weeks of pregnancy may be contributed by the foetus.

Significant sex differences in foetal pituitary and serum concentrations of both FSH and LH during 12–20 weeks of gestation have previously been reported (Clements et al. 1976; Kaplan & Grumbach 1976). It has also been suggested that these may be the result of feedback inhibition by the higher concentration of testosterone in male foetuses. If the foetus in the source of the increased levels of LH in amniotic fluid during pregnancy, it could be expected that a significant sex difference may also appear in amniotic fluid LH concentrations. In favour of this view point, is the present finding that amniotic fluid LH levels in women bearing male foetuses are significantly lower than those in women bearing female foetuses. Of interest is the clear demarcation in LH concentrations at 16 weeks of gestation.
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REFERENCES


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