Amniotic fluid concentrations of dimeric inhibins, activin A and follistatin in pregnancy

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Abstract

Objective: The feto-placental unit is the major source of circulating concentrations of inhibin A and activin A in human pregnancy. The aim of this study was to measure the amniotic fluid concentrations of inhibin A, inhibin B, activin A and follistatin in pregnancies bearing male and female fetuses.

Design and Method: Amniotic fluid samples collected by amniocentesis were stored at −20 °C. Dimeric inhibins, ‘total’ activin A and ‘total’ follistatin were measured using specific two-site enzyme immunoassays. Samples were assayed blindly and the information on fetal sex was obtained from the cytogenetics laboratory.

Results: Data show that amniotic fluid concentrations of inhibin A, inhibin B and activin A gradually increase with gestation whilst concentrations of follistatin are similar between weeks 15 and 20 of pregnancy. Mean amniotic fluid levels of inhibin A and inhibin B at 16 and 17 weeks gestation and mean activin A levels at 15 and 16 weeks gestation are considerably lower in pregnancies with male (n = 24) compared with female (n = 28) fetuses. Levels of follistatin are not different in the male and female fetal pregnancies at any studied gestation.

Conclusions: The results indicate that amniotic fluid contains high concentrations of inhibins (A and B), activin A and follistatin in early pregnancy suggesting that these hormones are produced by the fetal membranes and may be involved in the development of the fetus.

European Journal of Endocrinology 140 420–424

Introduction

Inhibins are glycoprotein hormones consisting of two dissimilar subunits. Inhibin A is an α-βA dimer and inhibin B is an α-βB dimer. Activins are homodimeric glycoprotein hormones. Activin A is a βA-βA dimer, activin AB is a βA-βB dimer and activin B is a βB-βB dimer. Follistatin is a structurally distinct single chain glycoprotein hormone with functional similarity to inhibin. Within the last decade follistatin has also been identified as a high affinity activin binding protein (1).

Classically, inhibins and activins were isolated and purified based on their effect on pituitary follicle-stimulating hormone (FSH) secretion. Inhibins inhibited pituitary FSH secretion and activins stimulated FSH secretion. Inhibins, activins and follistatins were initially purified and characterised from ovarian follicular fluid in different species. Extragonadal sources have also been identified for these proteins (2). Development of specific assays for inhibin A (3, 4), inhibin B (5), activin A (6), activin AB (7) and follistatin (8) has enabled us to measure the specific molecular form of dimeric protein.

Using these new specific assays, high concentrations of inhibin A (9) and activin A (10) have been measured throughout pregnancy. However, inhibin B concentrations in maternal circulation during pregnancy have been reported to be very low, with levels close to or at the detection limit of the assay (11, 12). Studies in early pregnancy support a feto-placental origin for inhibin A and activin A (13–15). Messenger RNA for inhibin α subunit has been identified in the syncytiotrophoblast and inhibin/activin βA and βB mRNAs have been identified in both cytotrophoblast and syncytiotrophoblast in the placenta (2). Immunocytochemical staining has also shown the presence of the α subunit, βA subunit and the βB subunit protein in syncytiotrophoblast (16, 17) and the decidua (18).

Recently two studies have reported amniotic fluid levels of inhibin A and inhibin B in normal (19) and abnormal pregnancies affected with Down’s syndrome (20). However, levels of activin A and follistatin have not been studied in the amniotic fluid in the second trimester. The aim of this study was to investigate the concentrations of inhibin A, inhibin B, activin A and follistatin in amniotic fluid of pregnancies bearing male and female fetuses in the second trimester.
Materials and methods

Amniotic fluids

Amniotic fluid samples (n = 146) were collected at amniocentesis performed for clinical diagnostic reasons. Amniotic fluid was centrifuged to remove any fetal cells and the supernatant was frozen at −20°C until assayed for inhibin A, inhibin B, activin A and follistatin. All samples analysed were from genetically normal fetal pregnancies. Samples were analysed blind and the information on fetal sex was subsequently obtained from the cytogenetics laboratory. The results of assays from female fetus bearing pregnancies and male fetus bearing pregnancies were analysed to study any difference in levels of inhibins, activin A and follistatin. For statistical analysis, samples were separated into male (n = 13, n = 28, n = 15, n = 7, n = 5) and female (n = 12, n = 24, n = 24, n = 10 and n = 8) fetus bearing pregnancies at 15, 16, 17, 18 and 20 weeks respectively. Gestational age was determined by ultrasound and the date of the last menstrual period.

Hormone assays

Inhibin A Amniotic fluid concentrations of dimeric inhibin A were measured in duplicate 10 μl aliquots as described elsewhere (4). The mean intra- and interassay coefficients of variation were 4.3% and 5.1% respectively. The minimum detection limit of the assay for human recombinant inhibin A (NIBSC, South Mimms, UK) was 2 pg/ml.

Inhibin B Amniotic fluid concentrations of dimeric inhibin B were measured in 10 μl duplicate aliquots using an enzyme immunoassay (EIA) as described in detail elsewhere (5). An in-house standard preparation (partially purified human follicular fluid) was standardised against human recombinant inhibin B (Genentech, San Francisco, CA, USA) and was used as the assay standard. The minimum detection limit of the assay for human recombinant inhibin B was 10 pg/ml. The mean intra- and interassay coefficients of variation were 6.2% and 7.2% respectively.

Follistatin Amniotic fluid concentrations of ‘total’ follistatin (follistatin 288) were measured in duplicate as previously described (8). The minimum detection limit of the assay for human recombinant follistatin standard was 20 pg/ml. The mean intra- and interassay coefficients of variation were < 10%. The assay cross reacts 9.9% with follistatin 315.

Activin A Amniotic fluid concentrations of ‘total’ activin A were measured using an EIA specific for ‘total’ activin A as described in detail elsewhere (6). The mean intra- and interassay coefficients of variation were 6.5% and 7.7% respectively. The minimum detection limit of the assay for human recombinant activin A (Genentech) was 50 pg/ml.

Statistical analysis

Unpaired Student’s t-tests were carried out to investigate the difference in amniotic fluid concentrations of the hormones between male and female fetus bearing pregnancies at a particular gestation. One-way analysis of variance with Bonferroni Dunn posthoc test was carried out to investigate the changes in levels across the gestations. Correlation analysis (Pearson correlation) was used to study the relationship between the hormones with increasing gestation time.

Results

Amniotic fluid levels of inhibin A increased with gestation from 15 to 18 weeks and then remained similar up to 20 weeks (Fig. 1a). Levels of inhibin A tended to be higher in female (0.68 ± 0.08 ng/ml; 1.19 ± 0.6 ng/ml respectively) than in male (0.55 ± 0.05 ng/ml; 0.75 ± 0.12 ng/ml respectively) bearing pregnancies at 16 and 17 weeks gestation, but this difference did not reach statistical significance (Fig. 1b and Table 1).

Inhibin B concentrations in the amniotic fluid increased progressively from 15 to 20 weeks (P < 0.001; ANOVA; Fig. 2a). Although levels of inhibin B also appeared higher in the amniotic fluid from female fetus bearing pregnancies (0.26 ± 0.04 ng/ml, 0.42 ± 0.06 ng/ml, 0.66 ± 0.24 ng/ml and 0.6 ± 0.17 ng/ml
respectively) than from male fetus bearing pregnancies 
(0.2 ± 0.04 ng/ml, 0.26 ± 0.07 ng/ml, 0.32 ± 0.09 ng/ 
ml and 0.41 ± 0.19 ng/ml respectively) from 16 to 20 
weeks, the difference was not statistically significant 
(Fig. 2b and Table 1).

Total activin A levels also rose slightly from 15 to 20 
weeks gestation (Fig. 3a) but with no statistical signifi-
cance. Activin A levels also appeared to be higher in female 
fetus bearing pregnancies (5.17 ± 0.62 ng/ml, 5.81 ± 0.81 ng/
ml respectively) than in male fetus bearing 
pregnancies (3.9 ± 0.06 ng/ml, 4.84 ± 0.39 ng/ml) at 
15 and 16 weeks gestation (Fig. 3b and Table 1) but 
the difference was not statistically significant.

Follistatin levels did not alter between 15 and 20 
weeks gestation (Fig. 4a). Levels of follistatin were also 
similar in male and female fetus bearing pregnancies 
(Fig. 4b and Table 1) at the studied gestations.

Correlation analysis showed that amniotic fluid 
concentrations of inhibin A and follistatin were not 
significantly correlated with any other parameters

| Table 1 Concentrations of inhibin A, inhibin B, activin A and follistatin in male and female bearing pregnancies from 15 to 20 weeks gestation. Results are means ± S.E.M. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                 | 15              | 16              | 17              | 18              | 20              |
| Inhibin A (ng/ml)               |                 |                 |                 |                 |                 |
| Female                          | 0.32 ± 0.06     | 0.68 ± 0.08     | 1.19 ± 0.6      | 0.68 ± 0.08     | 0.71 ± 0.12     |
| Male                            | 0.36 ± 0.06     | 0.55 ± 0.05     | 0.75 ± 0.12     | 0.55 ± 0.05     | 0.56 ± 0.3      |
| Inhibin B (ng/ml)               |                 |                 |                 |                 |                 |
| Female                          | 0.09 ± 0.02     | 0.26 ± 0.04     | 0.42 ± 0.06     | 0.66 ± 0.24     | 0.6 ± 0.17      |
| Male                            | 0.15 ± 0.04     | 0.2 ± 0.04      | 0.26 ± 0.07     | 0.32 ± 0.09     | 0.41 ± 0.19     |
| Activin A (ng/ml)               |                 |                 |                 |                 |                 |
| Female                          | 5.17 ± 0.62     | 5.8 ± 0.8       | 4.5 ± 0.48      | 5.8 ± 0.8       | 8.9 ± 1.7       |
| Male                            | 3.9 ± 0.06      | 4.84 ± 0.39     | 4.89 ± 0.62     | 7.39 ± 0.6      | 5.21 ± 2.4      |
| Follistatin (ng/ml)             |                 |                 |                 |                 |                 |
| Female                          | 1.8 ± 0.12      | 1.57 ± 0.12     | 1.48 ± 0.1      | 1.49 ± 0.19     | 1.4 ± 0.15      |
| Male                            | 1.63 ± 0.16     | 1.93 ± 0.15     | 1.62 ± 0.18     | 1.57 ± 0.6      | 1.36 ± 0.52     |

Figure 2 (a) Scatter plot of individual concentrations of amniotic fluid inhibin B at different gestations. (b) Mean ± S.E.M. concentrations of inhibin B in male (open bars) and female (solid bars) fetus bearing pregnancies. P<0.001 (ANOVA) – significant increase with gestation.

Figure 3 (a) Scatter plot of individual concentrations of amniotic fluid activin A at different gestations. (b) Mean ± S.E.M. concentrations of activin A in male (open bars) and female (solid bars) fetus bearing pregnancies.
In the first and second trimester placenta, positive
localisation of inhibin/activin subunits using specific
antibodies to the $\alpha$, $\beta_A$ and $\beta_B$ subunits in the placenta.
In the first and second trimester placenta, positive
staining for $\alpha$ and $\beta_A$ subunits was clearly observed in
the syncytial layer, with very faint staining for $\beta_B$
subunits (16). The epithelial layer of the amnion
showed an intense fluorescent staining for $\beta_B$ subunit
and positive signals were also observed for the $\alpha$ and $\beta_A$
subunits (21, 22). Maternal decidual cells stained with
both inhibin $\alpha$ and $\beta_B$ antisera showed a similar
localisation. Cells stained with $\beta_A$ subunit were sparse
and followed a distribution pattern different from that of
cells stained with $\alpha$ or $\beta_B$ antisera (18).

This study's data are consistent with other reports
showing that high concentrations of inhibins (19, 20)
and 'total' activin A (23) are present in the amniotic
fluid. In our study, we have investigated the levels of
inhibins (A and B), activin A and follistatin in the
amniotic fluid of male and female bearing pregnancies.

The follistatin assay used in this study essentially
detects follistatin 288 and cross reacts considerably
with follistatin 315 (9.9%). The amount of the different
molecular forms of follistatin in the amniotic fluid is not
known although follistatin 315 is reported to be the
major follistatin form in human circulation (24).
However, high concentrations (1–2 ng/ml) of follistatin
288 are present in amniotic fluid. This study shows that
follistatin levels tend to decrease in amniotic fluid
between 15 and 20 weeks gestation, in contrast to the
pattern seen for inhibins and activin A. However, there
is no significant correlation between follistatin and
activin A or inhibins.

Measurements in peripheral serum collected from
adult men have identified mainly inhibin B (25), activin
A (6) and follistatin (8) with inhibin A levels close to the
detection limit of the assay. In contrast, amniotic fluid
contains high concentrations of inhibin A, inhibin B,
activin A and follistatin whether the fetus is male or
female. However, it is very interesting that inhibin B
levels are high in the amniotic fluid and hardly detectable
in the maternal circulation indicating that the source of
these proteins in the maternal circulation is different
from the source in the amniotic fluid. It will be interesting
to investigate the levels of these proteins in the uterine
vein and compare it with maternal circulating levels.

High concentrations of inhibins, activin A and
follistatin in the amniotic fluid suggest a biological
function for these proteins in this fetal compartment in
pregnancy. Little is known about the physiological role
of these proteins in pregnancy although the maternal
circulating levels and amniotic fluid levels are higher in
pregnancy compared with the menstrual cycle.

Activin-A is reported to play a role in early embryo
development and mesoderm induction in other species.
However, there are no reported studies on the effect of
activin/inhibin on human embryo development (26).

In summary, amniotic fluid is a source of inhibin A,
inhibin B, activin A and follistatin 288 and 315 in
second trimester pregnancy. Levels of inhibin B pro-
gressively rise between 15 and 20 weeks gestation
whereas inhibin A, activin A and follistatin levels do not
change significantly. Levels of all proteins do not vary
significantly between pregnancies bearing male and
female fetuses.

Figure 4 (a) Scatter plot of individual concentrations of amniotic
fluid follistatin at different gestations. (b) Mean ± S.E.M. concentra-
tions of follistatin in male (open bars) and female (solid bars) fetus
bearing pregnancies.

Discussion

We have investigated the amniotic fluid concentrations
of inhibin A, inhibin B, activin A and follistatin from 15
to 20 weeks gestation and in male and female fetus
bearing pregnancies. This is the first study to report
activin A and follistatin levels in the amniotic fluid in
the second trimester of male and female bearing
pregnancies. Maternal serum concentrations of inhibin
A and activin A have been reported to be high in the
circulation throughout pregnancy (9, 10). However,
inhibin B levels are low and near the detection limit of
the assay (11, 12). In pregnancy, the major source
of inhibin A and activin A is the feto-placental unit
(13–15). However, the feto-placental source of inhibins
and activin A in maternal serum could be different from
the source of amniotic fluid inhibins and activin A
because inhibin B is not detectable in maternal
circulation but is present in high quantities in the
amniotic fluid.

Studies have been carried out on the cellular
localisation of inhibin/activin subunits using specific
antibodies to the $\alpha$, $\beta_A$ and $\beta_B$ subunits in the placenta.
In the first and second trimester placenta, positive
staining for $\alpha$ and $\beta_A$ subunits was clearly observed in
the syncytial layer, with very faint staining for $\beta_B$
subunits (16). The epithelial layer of the amnion
means. Inhibin B levels were significantly correlated
with activin A levels ($r = 0.2$, $P < 0.02$).
Acknowledgements

The authors thank the Cytogenetics Department at the Oxford Radcliffe Trust for the karyotype results, Genentech Inc. for the human recombinant activin A standards and the National Institute for Biological Standards for the recombinant inhibin A standards. S.M. acknowledges the Wellcome Trust for financial support.

References

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