

## Source of circulating levels of inhibin A, pro alpha C-containing inhibins and activin A in early pregnancy

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It is now established that the glycoprotein hormone inhibin is produced by primate granulosa cells, corpus luteum and trophoblast of human placenta. This study was designed to investigate the major source of inhibins and activin A in early pregnancy using a novel panel of assays with high specificity and sensitivity. A total of 12 women (aged 20–35 years) with singleton pregnancy undergoing first trimester (group 1: 6–8; group 2: 8–10; group 3: 10–12 weeks of gestation) termination of pregnancy (TOP) was recruited for the study. Blood samples were taken before TOP, every 15 min for the first hour and hourly for the next 3 h after TOP (total of 4 h of measurements). Circulating concentrations of inhibin A, pro alpha C, activin A, human chorionic gonadotrophin (HCG), oestradiol and progesterone were higher in early pregnancy than at any stage of the menstrual cycle. Peripheral concentrations of inhibin A and activin A were significantly decreased within the first hour in all three groups and gradually decreased to even lower concentrations within the study period. Pro alpha C concentrations decreased within the first hour and then remained unaltered during the next 3 h. Similarly, HCG, oestradiol and progesterone concentrations in circulation decreased substantially within 4 h of TOP. Correlation analyses showed a significant positive correlation ( $P < 0.001$ ) between inhibin A, activin A, HCG, and oestradiol concentrations throughout the study period. In summary, this study shows that the fetoplacental unit is the major source of increased circulating concentrations of inhibin A in early pregnancy. Activin A is produced by the fetoplacental unit and the corpus luteum. Pro alpha C-containing inhibins are mainly secreted by the corpus luteum in early pregnancy.

**Key words:** activin/fetus/inhibin/placenta/pregnancy

### Introduction

Inhibins are heterodimeric glycoprotein hormones consisting of an  $\alpha$ -subunit and a  $\beta_A$  (inhibin A) or a  $\beta_B$  subunit (inhibin B) linked by disulphide bonds. Activins are homodimers consisting of  $\beta_A\beta_A$  (activin A),  $\beta_A\beta_B$  (activin AB) and  $\beta_B\beta_B$

(activin B) subunits linked by disulphide bonds. It is now well established that inhibin is synthesized and secreted by primate granulosa cells and corpus luteum. Several studies have shown that immunoreactive inhibin is synthesized and secreted by the human placenta (McLachlan *et al.*, 1986; Mayo *et al.*, 1986; Petraglia *et al.*, 1987; Meuriner *et al.*, 1988). Previous studies have reported an increase in concentrations of immunoreactive inhibin during first trimester pregnancy and a further rise at term (McLachlan *et al.*, 1987; Yohkaichiya *et al.*, 1989; Abe *et al.*, 1990; Tabei *et al.*, 1991). However, in all the above studies immunoreactive inhibin was measured using radioimmunoassays which cross-react extensively with monomeric inhibin  $\alpha$ -subunit.

Recent development of specific and sensitive enzyme immunoassays for dimeric inhibin A (Groome *et al.*, 1994; Muttukrishna *et al.*, 1994), dimeric inhibin B (Groome *et al.*, 1996), pro alpha C (Groome *et al.*, 1995) and 'total' activin A (Knight *et al.*, 1996) has facilitated the measurement of circulating concentrations of bioactive dimeric inhibins and activin A. Evidence on circulating concentrations of dimeric inhibin A during the normal menstrual cycle (Groome *et al.*, 1994; Muttukrishna *et al.*, 1994) confirms the production of inhibin A by the granulosa cells and the corpus luteum. Studies on circulating concentrations of dimeric inhibin A throughout pregnancy (Muttukrishna *et al.*, 1995) also confirm that high concentrations of dimeric inhibin A are in circulation during human pregnancy.

Placental trophoblasts express inhibin  $\alpha$ ,  $\beta_A$  and  $\beta_B$  subunit mRNA (Northern blot analysis) and produce immunoreactive inhibin (immunocytochemistry) at different stages of pregnancy (Baird and Smith, 1993). In-vitro studies have also confirmed the production of immunoreactive inhibin by the cytotrophoblast of the human placenta (McLachlan *et al.*, 1986; Mayo *et al.*, 1986; Petraglia *et al.*, 1987; Meuriner *et al.*, 1988).

Immunoreactive inhibin concentrations rise after conception in early pregnancy (Tovanabutra *et al.*, 1993). Whilst the corpus luteum is a major site of inhibin production during the luteal phase in a normal menstrual cycle (Illingworth *et al.*, 1991), there is conflicting evidence on the source of inhibin in early pregnancy. McLachlan *et al.* (1987) reported that concentrations of immunoreactive inhibin in circulation are similar in normal pregnant women and women without a functional ovary (involved in a donor oocyte programme). In contrast, Yohkaichiya *et al.* (1991) reported that although immunoreactive inhibin concentrations showed a similar pattern in both normal pregnant women and women involved in a donor oocyte in-vitro fertilization (IVF) programme, the concentrations in women without a functional ovary were much less than in normal pregnant women with a functional

ovary. Both studies are based on conventional radioimmunoassay for inhibin which strongly cross-reacts with free-monomeric  $\alpha$ -subunit. One method for studying the contribution of the placenta to the circulating concentration of a hormone in early pregnancy is to determine the rate of clearance following the removal of the placenta during pregnancy termination.

The present study was designed to investigate the source of different forms of inhibins and 'total' activin A during early pregnancy by measuring the changing concentrations of inhibins and activin A in patients undergoing first trimester termination of pregnancy (TOP) over a period of 4 h.

## Materials and methods

### Patients and sample collection

Three groups each of four women ( $n = 12$ ) with singleton pregnancy (group 1: 6–8 weeks; group 2: 8–10 weeks; group 3: 10–12 weeks) undergoing TOP were recruited in the gynaecology clinic for the study. The sampling protocol was approved by the Central Oxford Research Ethics Committee and written informed consent was obtained from the patients. Normal cycle samples were obtained from five cycling women during the follicular phase (day 5–8) and luteal phase (day 19–23).

Cervical pre-treatment with a prostaglandin E1 analogue (16,16-dimethyl-*trans*-d-2-PGE-methylester: Cervagem pessary; May and Baker Pharmaceuticals, Eastbourne, Sussex, UK) was administered 2–3 h before TOP. In all patients TOP procedure was carried out under general anaesthesia.

One blood sample (5 ml) was taken immediately before the TOP procedure. Blood samples were then taken every 15 min for 1 h and then hourly up to 4 h after TOP. One 5 ml blood sample was taken from normally cycling women during follicular phase (day 5–8) and during luteal phase (day 19–23). Blood was collected in EDTA and plasma was separated and stored at  $-20^{\circ}\text{C}$  for hormone assays.

### Hormone assays

#### Inhibin A

Plasma concentrations of dimeric inhibin A were measured in duplicate 25  $\mu\text{l}$  and 50  $\mu\text{l}$  aliquots as described elsewhere (Muttukrishna *et al.*, 1994). The mean intra- and interassay variations were 4.3 and 5.1% respectively. Minimum detection limit of the assay for human recombinant inhibin A (kindly provided by Dr M.Rose, National Institute for Biological Standards, Potters Bar, Herts, UK) was 2 pg/ml.

#### Pro alpha C

Plasma concentrations of pro alpha C were measured in duplicate 25  $\mu\text{l}$  and 50  $\mu\text{l}$  aliquots as described before (Groome *et al.*, 1995) with some modifications. Minimum detection limit of the assay for pro alpha C standard (N.P.G.) was 5 pg/ml. The mean intra- and interassay variations were 6.8 and 9.1% respectively.

#### Activin A

Plasma concentrations of dimeric activin A were measured using a recently developed enzyme immunoassay specific for 'total' activin A as described in detail elsewhere (Knight *et al.*, 1996). The mean intra- and interassay variations were 6.5 and 7.7% respectively. The minimum detection limit of the assay for human recombinant activin A (Genentech, San Francisco, CA, USA) was 50 pg/ml.

#### Oestradiol, progesterone and HCG

Plasma concentrations of oestradiol, progesterone and human chorionic gonadotrophin (HCG) were measured using Immulite chemiluminescent assay kits (Diagnostic Products Corporation, Los Angeles,

CA, USA). The detection ranges of oestradiol, progesterone and HCG assays were 0.073–7.32 nmol/l, 0.73–127 nmol/l and 5–5000 mIU respectively. All samples were assayed simultaneously and the mean intra-assay variation for oestradiol, progesterone and HCG was  $<10\%$  for all three assays.

### Statistical analysis

One-way analysis of variance was carried out to investigate the changing concentrations of different hormones with time within-subject variables. Spearman's correlation analysis was carried out to investigate the relationship between different hormones. All statistical analyses were carried out using Prism statistical package (GraphPad Software Inc., San Diego, CA, USA) using 95% confidence interval limit.

## Results

Peripheral concentrations of inhibin A, activin A, and pro alpha C varied in patients before the TOP procedure. The variation of hormone concentrations in patients in relation to time of gestation was comparable with previous studies (Muttukrishna *et al.*, 1995;1996; Illingworth *et al.*, 1996). However, the pattern of clearance of all three hormones was similar in all patients irrespective of the period of gestation in all three groups before the TOP. Insertion of prostaglandin analogue  $\sim 2$  h before TOP did not significantly alter the concentrations of inhibins and activin A (unpublished observations, S.M.).

### Changing concentrations of inhibins

#### Inhibin A

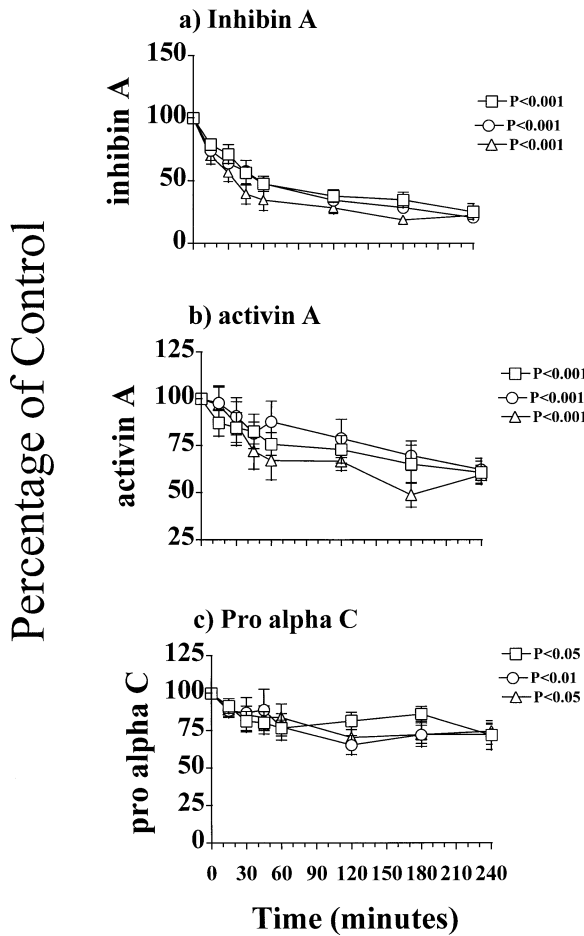
Circulating concentrations of dimeric inhibin A fell significantly for 4 h ( $P < 0.001$ ) in all three groups of patients after the removal of the fetoplacental unit from the patients (Figure 1a). Almost 50% of inhibin A disappeared from the circulation within the first hour, and concentrations gradually declined by a further 50% during the next 3 h. Circulating inhibin A concentrations were  $\sim 4$ -fold higher than luteal phase concentrations before TOP (Table I). Concentrations were similar to those in luteal phase at the end of the study period (Table I), indicating the presence of a viable corpus luteum 4 h after TOP.

#### Pro alpha C-containing inhibins

Peripheral concentrations of pro alpha C decreased significantly in all groups of subjects within the first 60 min ( $\sim 25\%$ ,  $P < 0.05$ ) and then remained almost unaltered throughout the rest of the study period (Figure 1c).

### Changing concentrations of 'total' activin A

Concentrations of activin A fell significantly ( $P < 0.01$ ) after TOP. A decrease of  $\sim 20\%$  occurred in groups 1 and 2 and of  $\sim 30\%$  in group 3 within the first hour. Activin A concentrations gradually declined to  $\sim 50\%$  of control in all three groups in the further 3 h period (Figure 1b). Peripheral concentrations of activin A at the end of the study period in all three groups were marginally higher than normal cycle luteal concentrations (Table I).



**Figure 1.** Data presented are normalized means ( $\pm$ SEM) of (a) inhibin A, (b) 'total' activin A and (c) pro alpha C concentrations in peripheral plasma before and after the termination of pregnancy (TOP) (concentration before TOP is 100%; see Table I for absolute values). Time 0 = immediately before TOP; group 1: 6–8 weeks; group 2: 8–10 weeks; group 3: 10–12 weeks gestation ( $n = 4$  in each group).  $\square$ , group 1;  $\circ$ , group 2;  $\triangle$ , group 3.

**Changing concentrations of other hormones**

**HCG**

Peripheral concentration of HCG fell significantly for 4 h ( $P < 0.001$ ) with time after TOP in all three groups of patients. However, the rate of disappearance of HCG from circulation was markedly less than those of the inhibins and activin A. In all three groups ~65% of HCG remained in circulation at the end of the study period (Figure 2a).

**Oestradiol**

Circulating concentrations of oestradiol fell significantly for 4 h ( $P < 0.001$ ) after the removal of the fetoplacental unit in all groups of patients. Concentrations fell by ~50% within the first hour in groups 1 and 2 and gradually fell to 30% of the initial concentration in the following 3 h period. In group 3 patients, oestradiol concentration fell to ~30% of control within the first hour and further declined to ~20% of the control within the study period (Figure 2c).

**Progesterone**

Plasma concentrations of progesterone fell significantly ( $P < 0.01$ ) by ~30% within the first hour in all three groups and remained almost unaltered during the rest of the study period in group 1 patients. Progesterone concentrations continued to fall by ~20% in groups 2 and 3 within the study period.

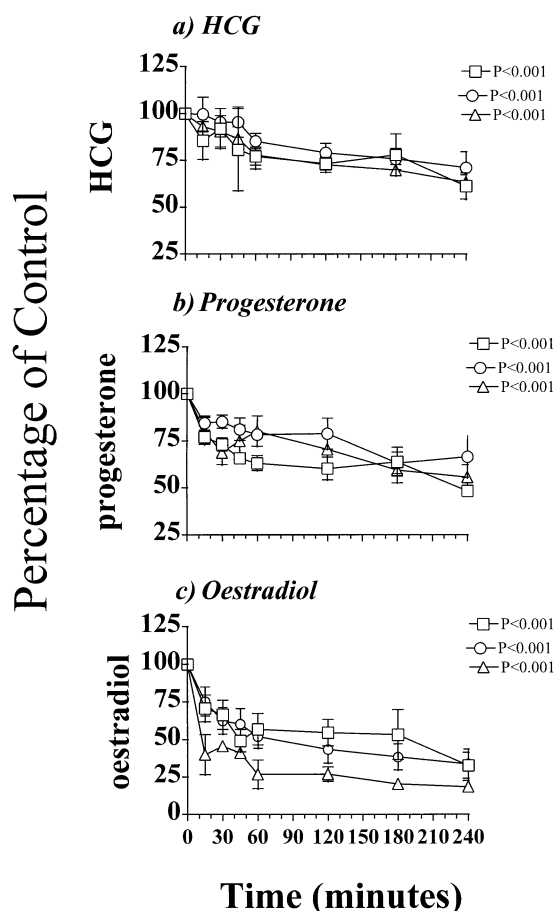
**Relationship between hormones**

Correlation analysis of combined data from all three groups showed that peripheral concentrations of inhibin A were significantly positively correlated with activin A ( $r = 0.52, P < 0.001$ ), oestradiol ( $r = 0.72, P < 0.001$ ) and HCG ( $r = 0.53, P < 0.001$ ) during the study period. Serum concentrations of activin A were significantly correlated with oestradiol ( $r = 0.37, P < 0.001$ ) and HCG ( $r = 0.42, P < 0.001$ ). Concentrations of pro alpha C were not correlated with those of other hormones measured. Serum HCG concentrations were correlated with oestradiol ( $r = 0.72, P < 0.001$ ).

**Table I.** Changes in circulating hormone concentrations

	Inhibin A (pg/ml)	Pro alpha C (pg/ml)	Activin A (pg/ml)	Progesterone (nmol/l)	Oestradiol (nmol/l)
FP ( $n = 5$ )	8.98 $\pm$ 0.18	246 $\pm$ 40.3	163 $\pm$ 15	2.67 $\pm$ 0.41	0.26 $\pm$ 0.03
LP ( $n = 5$ )	54 $\pm$ 0.7	571.1 $\pm$ 100	300 $\pm$ 57	33.2 $\pm$ 3.6	0.59 $\pm$ 0.11
T1					
Group 1 ( $n = 4$ )	181.6 $\pm$ 38.6	847 $\pm$ 116.2	721.9 $\pm$ 91.5	68 $\pm$ 8.3	4.42 $\pm$ 1.3
Group 2 ( $n = 4$ )	274.8 $\pm$ 53.4	492 $\pm$ 100	924.3 $\pm$ 270	61.25 $\pm$ 8.34	4.31 $\pm$ 1.1
Group 3 ( $n = 4$ )	262.8 $\pm$ 99.2	520.3 $\pm$ 109	889.5 $\pm$ 152.5	88.6 $\pm$ 9.98	7.4 $\pm$ 1.4
T2					
Group 1 ( $n = 4$ )	64.13 $\pm$ 20	572 $\pm$ 160.2	454 $\pm$ 85	41.8 $\pm$ 7.6	1.46 $\pm$ 0.31
Group 2 ( $n = 4$ )	55.4 $\pm$ 11.4	354.9 $\pm$ 71	566 $\pm$ 159	38.23 $\pm$ 3.9	1.12 $\pm$ 0.28
Group 3 ( $n = 4$ )	49.4 $\pm$ 9	383.5 $\pm$ 76	510.9 $\pm$ 62	50.3 $\pm$ 9.22	1.3 $\pm$ 0.23

Values are mean ( $\pm$ SEM) plasma concentrations. FP = mid-late follicular phase; LP = mid-late luteal phase; T1 and T2 = first trimester pregnancy before and 4 h after termination respectively; groups 1, 2 and 3 = gestational weeks 6–8, 8–10 and 10–12 respectively.



**Figure 2.** Data presented are normalized means ( $\pm$ SEM) of (a) HCG, (b) progesterone and (c) oestradiol concentrations in peripheral plasma before and after the TOP (concentration before TOP is 100%; see Table I for absolute values). Time 0 = immediately before TOP; group 1: 6–8 weeks; group 2: 8–10 weeks; group 3: 10–12 weeks gestation ( $n = 4$  in each group). See Figure 1 for experimental details.

## Discussion

This study demonstrates that the rise in circulating concentrations of dimeric inhibin A in early pregnancy results mainly from secretion by the fetoplacental unit. All three groups of patients from weeks 6 to 12 had similar clearance pattern and concentrations at the end of the study period. Recently Rombauts *et al.* (1996) have reported higher concentrations of inhibin A in early IVF pregnancies compared to early normal pregnancies and concluded that there is an ovarian source for inhibin A in early pregnancy. Apart from the increased number of corpora lutea, concentrations of inhibin A in IVF pregnancies could have been altered because these patients were treated with exogenous HCG until day 12 for luteal support and were given progesterone up to week 14 of the pregnancy. In contrast, our data were derived from pregnancies after spontaneous conception, and without 'luteal support' with HCG or progesterone, and show that the corpus luteum secretes normal luteal concentrations of inhibin A and not higher concentrations in early pregnancy.

There is evidence for higher circulating concentrations of inhibin A (Muttukrishna *et al.*, 1995) and activin A

(Muttukrishna *et al.*, 1996) throughout pregnancy. Consistent with our previous observation, peripheral concentrations of both inhibin A (Groome *et al.*, 1994; Muttukrishna *et al.*, 1994) and activin A (Muttukrishna *et al.*, 1996) were higher in this group of pregnant women compared to the normal cycle (Table I). However, the physiological role of inhibins and activins in pregnancy is unknown. In-vitro studies using cultured placental trophoblasts suggest a possible autocrine/paracrine role(s) for inhibin and activin on regulating the secretion of placental HCG and progesterone (Petraglia *et al.*, 1989). Immunoreactive inhibin concentrations have been suggested to be a marker of hydatidiform mole (Yohkaichiya *et al.*, 1989). Immunoreactive inhibin (Van Lith *et al.*, 1992; Spencer *et al.*, 1993) and dimeric inhibin A concentrations have also been reported to be elevated in the second trimester (Cuckle *et al.*, 1995; Wald *et al.*, 1996; Wallace *et al.*, 1996) of pregnancies affected with Down's syndrome and high concentrations of pro alpha C are reported to be an indicator of viable pregnancy (Illingworth *et al.*, 1996). These data show that inhibin A concentrations fall within 4 h of TOP to luteal phase concentrations (Table I), strongly suggesting that the rise in circulating inhibin A seen in early pregnancy results from the fetoplacental unit.

Both inhibin A and activin A had a bi-exponential pattern of fall during the study period. These data also show that the time taken to clear half the elevated concentration of inhibin A (half-life) from peripheral circulation is  $\sim$ 45 min. The inhibin B concentrations are unaltered in this study (data not shown), supporting the work of Illingworth *et al.* (1996), showing that inhibin B concentration in circulation is unaltered in early pregnancy.

Similar to inhibin A, activin A concentrations fell to  $\sim$ 50% of the pre-TOP level within 4 h after TOP in all three groups studied, suggesting the fetoplacental unit is also a source of activin A in early pregnancy. However, activin A concentrations 4 h after TOP were higher than in luteal phase concentrations (Table I), indicating an ovarian contribution of activin A in early pregnancy and/or a slow metabolic clearance for activin A. Yamoto *et al.* (1991) reported the presence of inhibin/activin subunits in the corpus luteum during human pregnancy, suggesting a possibility of luteal activin A production during pregnancy as seen in the present study. Human ovary is also reported to develop many antral follicles (Govan, 1968) during pregnancy. These antral follicles may also produce activin A in early pregnancy as antral follicles are reported to express relatively more inhibin  $\beta$ -subunits (Hillier, 1991).

Pro alpha C enzyme immunoassay is likely to cross-react with higher molecular weight forms of inhibins containing the pro alpha region (Groome *et al.*, 1995). Thus it is a cumulative measurement of monomeric pro alpha C subunit and pro alpha-containing inhibins. The present data show that concentrations of pro alpha C slightly declined within the first hour after TOP and remained unaltered during the rest of the study period in all three groups, suggesting that the maternal corpus luteum is the major source of pro alpha C and pro alpha-containing inhibins. Four hours after TOP, the concentrations of activin A, pro alpha C, oestradiol and progesterone (Table I) remained higher than normal cycle concentrations.

Previous studies using the conventional inhibin radio-immunoassays have reported a contribution of both the ovary and the placenta in pregnancy (McLachlan *et al.*, 1987; Kettle *et al.*, 1991; Yokkaichiya *et al.*, 1991). It is evident from this study and other reports (Groome *et al.*, 1996; Illingworth *et al.*, 1996) that different molecular forms of inhibins have different source and possibly have different roles in pregnancy. Discrepancy between our observation and previous observations could be due to the specificity of the inhibin A enzyme immunoassay used in our study. The conventional radioimmunoassay used in the previous studies measures dimeric inhibins and the monomeric  $\alpha$ -subunit.

Correlation analysis showed a significant correlation between the steroids, inhibin A, activin A and HCG disappearance curves. The disappearance curve of inhibin A in this study follows the pattern of disappearance of immunoreactive inhibin at term delivery of the placenta (Kettle *et al.*, 1991). However, in contrast to the study at delivery, the disappearance of steroids from maternal circulation took substantially longer than inhibin A, further supporting a maternal contribution of steroids in early pregnancy. However, at the end of the study period peripheral concentrations of progesterone remained almost similar to normal luteal phase concentrations, providing evidence for the presence of a functioning corpus luteum 4 h after TOP. Although concentrations of HCG varied during the experimental period, the data show a slow metabolic clearance rate for HCG compared to inhibins and steroids.

Collectively, this study provides evidence for the fetoplacental unit being a source of raised concentrations of inhibin A, activin A and pro alpha C early in pregnancy.

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