Concentrations of activin A, inhibin A and follistatin in human amnion, choriodecidual and placental tissues at term and preterm

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Abstract

To investigate labour-associated changes in production of activin and related hormones by gestational tissues we prepared extracts from amnion, choriodecidual and placental tissues delivered at term before labour (TNL; $n=15$), at term after spontaneous labour (TSL; $n=15$) or preterm (PTD; $n=31$) and measured concentrations of inhibin A, activin A and follistatin by ELISA. Activin concentrations in placental tissues were significantly (Mann–Whitney U-test; $P<0.05$) elevated with term labour (pg/mg protein, median; 1313 vs 2591), but in the PTD tissues concentrations were lower than those delivered spontaneously at term (3650 vs 2649). Inhibin concentrations also increased with term labour in the placenta (480 vs 686), but paradoxically decreased in amnion (188 vs 64) and choriodecidual (657 vs 358). Little or no significant changes in follistatin concentrations were

observed. Concentrations of all three proteins were significantly correlated between amnion and choriodecidual tissues, and were significantly correlated with each other in most tissues (Spearman's ranked correlation; $P<0.05$). The activin:inhibin ratio in term amnion and choriodecidual tissues was increased 2 to 3-fold ($P<0.0005$ by Mann–Whitney U-test) after term labour, with similar trends also observed in the activin:follistatin ratio in placental tissue. These data suggest that a modest increase in placental activin and inhibin production may occur with labour at term. In addition, an increase in activin bioactivity may occur with labour, potentiating any paracrine effects of activin during parturition. The data, however, do not support an association between increased intrauterine activin biosynthesis and preterm delivery.

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Introduction

The human placenta synthesises and secretes numerous paracrine and autocrine factors during pregnancy, the roles of many of which have not been clearly defined. Among these are activin, its antagonist inhibin, and its binding protein follistatin (Petraglia 1997). Described recently as a 'new cytokine' (Yu *et al.* 1996), activin is more traditionally viewed as a reproductive hormone/growth factor, with pleiotrophic actions in a wide range of tissues. Both activin and inhibin are members of the transforming growth factor β superfamily (TGF- β) (Massague 1990, de Kretser 1993), while follistatin is unrelated in structure or sequence (Esch *et al.* 1987). Activin and inhibin subunits are synthesised by the human placenta and extraplacental membranes (Petraglia *et al.* 1990, 1991, 1994a, Minami *et al.* 1992, de Kretser *et al.* 1994, Loveland *et al.* 1996, Petraglia 1997). The reported rise in activin A and inhibin A in maternal serum that occurs through pregnancy, particularly in late gestation (Petraglia *et al.* 1993a, 1994b,

Muttukrishna *et al.* 1995, Knight *et al.* 1996, Wallace *et al.* 1997, Fowler *et al.* 1998, O'Connor *et al.* 1999), is believed to be a consequence of a rise in placental production, although this has not been proven experimentally and supportive evidence is not conclusive (Minami *et al.* 1992). Various forms of follistatin have been measured in maternal circulation and these have also been reported to rise with advancing gestation (Wakatsuki *et al.* 1996, Woodruff *et al.* 1997, Evans *et al.* 1998, Fowler *et al.* 1998), probably as a consequence of increased placental output (de Kretser *et al.* 1994, Yokoyama *et al.* 1995).

Amniotic fluid concentrations of activins and inhibins rise following labour (Petraglia *et al.* 1993a, 1997), suggesting a possible elevation of production by the foetal membranes. This possibility is supported by recent findings of increased activin A mRNA expression in amnion and chorion with term and preterm labor (Petraglia *et al.* 1997). Additionally, concentrations of free activin A in the maternal circulation of women presenting with preterm labour are elevated with respect to normal term deliveries

(Petraglia *et al.* 1995), an observation consistent with the reported increase in activin A pulse amplitude found in this group (Gallinelli *et al.* 1996). One of the most common causes of preterm labour and delivery is believed to be intrauterine infection (Mitchell *et al.* 1991, Romero *et al.* 1994). We have recently defined interleukin-1 β and tumour necrosis factor- α as potent stimulators of activin A production by gestational tissues, drawing a putative link between infection-driven preterm labour and elevated intrauterine activin production (Keelan *et al.* 1998).

To assess changes in activin A, inhibin A and follistatin production in the gestational membranes and placenta with labour at term and preterm, we measured the content of these proteins in soluble extracts prepared from tissues collected from amnion, choriodecidual and placental tissues from pregnancies delivered preterm or at term, before or after labour.

Materials and Methods

Materials

Avidchrome hydrazide-coated plates for the activin A assay were purchased from Unisyn Technologies, San Diego, CA, USA, while FluoroNunc plates for the inhibin assay were supplied by Nalge Nunc International, Roskilde, Denmark. 4-methyl-umbelliferyl phosphate (MUP) was purchased from Molecular Probes Inc., Eugene, OR, USA. Pepstatin-A and phenylmethylsulfonylfluoride (PMSF) were supplied by Sigma Chemical Co., St Louis, MO, USA. Sodium dodecyl sulphate (SDS) and bovine serum albumin (BSA) were supplied by Serva, Boehringer Ingelheim Bioproducts Partnership, Heidelberg, Germany.

Tissues and clinical details

Maternal consent for the collection of placental tissues was obtained according to the guidelines of the Auckland Human Ethics Committee. Placentae were collected and processed within 1 h of delivery. Placentae were obtained from women who were delivered by Caesarean section (indication: previous Caesarean section or malpresentation) at term before the onset of labour (TNL; $n=15$), after uncomplicated vaginal delivery at term following spontaneous onset of labour (TSL; $n=15$), or preterm (PTD; $n=31$). Tissues derived from preterm deliveries were scored according to the presence of evidence of possible intrauterine infection (two or more of the following: maternal fever, maternal or foetal tachycardia, foul-smelling liquor, uterine tenderness, histological chorioamnionitis or neonatal sepsis) (Gibbs *et al.* 1992).

Tissue extraction

After manually stripping the amnion from the choriodecidual, sections of the membranes ($\sim 2\text{ cm}^2$) were

cut, washed briefly in cold PBS to remove excessive amounts of blood, and rapidly frozen in liquid nitrogen. At the same time a small piece of villous placental tissue ($\sim 1\text{ cm}^3$) was dissected free of maternal decidua from the placental bed, and similarly frozen after a brief wash in PBS. When ready for processing, samples were thawed in 2 ml lysis buffer (0.05 M Tris-HCl pH 6.8, 2 mM EDTA, 100 g/l sucrose, 50 $\mu\text{g}/\text{ml}$ pepstatin-A, 0.1 g/l PMSF and 0.1 g/l sodium azide) at 4 °C. They were initially processed using a polytron homogeniser for 15 s on ice, followed by sonication using a Branson sonicator for 30 s. A 1 ml aliquot of each homogenate was taken, centrifuged for 7 min at 10 000 g, and the supernatants removed and stored frozen at $-70\text{ }^\circ\text{C}$ prior to assay. A small aliquot of each supernatant was taken and diluted 20-fold in 0.1 N NaOH for protein assay by the bicinchoninic acid (BCA) method (Redinbaugh & Turley 1986). The assay had a working range of $\sim 10\text{--}1000\text{ }\mu\text{g}/\text{ml}$ calibrated against BSA.

Immunoassays

Activin A, inhibin A and follistatin were measured by enzyme-amplified ELISA essentially as detailed previously (Muttukrishna *et al.* 1995, Knight *et al.* 1996, Evans *et al.* 1998). The assays employed a dissociation step and hence measured total (i.e. bound and free) activin A, inhibin A and follistatin. The inhibin A assay was modified from that originally described to employ FluoroNunc ELISA plates passively-coated with capture antibody and incorporation of an enzyme-catalysed fluorescence substrate MUP. It was standardised against the First International Standard for inhibin with an assigned potency of 75 U/ml (1000 pg/ml) as the highest standard (Rose & Gaines Das 1996). All tissue extracts were assayed at a single dilution in lysis buffer, which was also used as the standard diluent. The activin A assay performed with an interassay precision (coefficient of variation) of 9% ($n=5$) and a sensitivity of $\sim 25\text{ pg}/\text{ml}$. The interassay precision of the inhibin A assay was 6% ($n=4$) and its limit of detection was $\sim 10\text{ pg}/\text{ml}$ (0.75 U/ml). The follistatin assay had an interassay precision of 6% ($n=4$) and a sensitivity of $\sim 20\text{ pg}/\text{ml}$. The recovery of activin A, inhibin A and follistatin from all three tissue types ranged from 83.8 to 101.4%.

Statistical analysis and representation of data

Tissue concentrations of activin A, inhibin A and follistatin are expressed as pg/mg protein. Differences between the absolute and relative concentrations of analytes in the various gestational categories were determined by Mann-Whitney U-test for non-parametric data. A P value <0.05 was considered to be significant. Spearman's ranked correlations were performed to determine relationships between levels of the various hormones in the tissues

Table 1 Clinical and demographic characteristics relating to the tissues obtained after preterm delivery

#	Gestational age at delivery (week(s))	Singleton or twin	Time in labour (hour)	Deliver mode	Indication for CS	Other conditions	Placental histology	Clinical signs of infection	Tocolysis	Antibiotics	Weight at birth (g)
1	30	T	28	V			ND		N	Aug	1330
2	30	T	28	V			ND		N	Aug	1450
3	26	S	36	CS	MP		-VE	NS	N	Aug	770
4	33	S	12	V			ND		N	None	2270
5	27	S	84	CS	MP		ND	FT, NS	S	None	800
6	31	S	11.5	V		PROM	ND	FT, MT, NS	S	None	1590
7	25	S	20	CS	MP		-VE		S	Aug	765
8	31	S	4	CS	APH	PROM/IUGR	ND		N	Aug	1136
9	33	S	5.5	V		PROM/IUGR	ND		N	None	1950
10	25	S	20	V		PROM/IUGR	ND		N	Aug	540
11	32	S	14	V			-VE		N	None	1750
12	33	S	13	V		PROM	ND	UT, NS	S	None	1775
13	25	S	84	V		PROM	ND		S	Aug	890
14	32	S	15	CS	Unknown		-VE		N	None	2800
15	31	T	3	CS	APH	PROM	ND		N	None	1730
16	31	T	3	CS	APH	PROM	ND		N	None	1795
17	28	S	14	V		PROM	-VE	FT, MF	S	Ceph	1400
18	34	S	6.5	V		PROM	ND		N	None	2360
19	31	S	2	CS	MP	PROM	ND		S	None	1850
20	31	S	55	V		PROM	ND	NS	S	None	1705
21	30	T	9	V	FTS	PROM	ND	NS	S	Aug	1610/1150
22	29	S	15	CS	FD	PROM	+VE	BS, NS	S	Aug	1190
23	32	T	0	CS	PROM	PROM	ND		N	Aug	2375
24	32	T	0	CS	PROM	PROM	ND		N	Aug	755
25	24	T	38	V		PROM	+VE	MF, FT, NS	S	Amox	550/585
26	26	S	38	V			ND	NS	S	None	705
27	32	S	21	V		PE	ND		S	Aug	1580
28	26	T	35	V		PROM	ND		S	Aug	745
29	26	T	35	V		PROM	ND		S	Aug	720
30	33	S	56	V			ND	NS	S	None	1990
31	33	S	17.5	V		PROM	ND		N	Aug	2555

S, singleton; T, twins; V, vaginal delivery; CS, Caesarean section; MP, malpresentation; APH, ante-partum haemorrhage; FTS, foetal transfusion syndrome; FD, foetal distress; PROM, premature rupture of membranes; IUGR, intrauterine growth restriction; PE, pre-eclampsia; ND, not determined; S, salbutamol; Aug, augmentin; Amox, amoxicillin; Ceph, cephalosporin; FT, foetal tachycardia; MT, maternal tachycardia; MF, maternal fever; NS, neonatal sepsis; BS, positive placental bacterial swab.

studied. Due to the non-availability of gestational age-matched normal controls, data from tissues delivered preterm were compared with those delivered at term.

Results

The gestational age at delivery for both term groups (TNL and TSL) ranged from 37 to 42 weeks. All were singleton pregnancies with the exception of one twin birth in the TNL group. There was no clinical evidence of intra-uterine infection in any of the term pregnancies. The gestational age at delivery in the PTD group ranged from 24 to 34 weeks (Table 1). In this group, 65% (20/31) were delivered vaginally. Of the remainder, ten were delivered by emergency Caesarean in labour (duration of labour, 2–84 h) and one by Caesarean section before labour after preterm premature rupture of the membranes. Ten sets of the PTD tissues (32%) were from twin pregnancies. Intravenous salbutamol was administered to 52% (16/31) of the preterm deliveries, while 55% (17/31) received antibiotics; all received antenatal corticosteroids. Spontaneous rupture of membranes occurred in 55% (17/31). Six PTD pregnancies (19%) were designated as infection positive (PTD+ve; #5, 6, 13, 17, 22 and 25, Table 1). Of these, three had placental histology performed of which two had histologically confirmed chorioamnionitis.

Activin A, inhibin A and follistatin were detectable in all samples of amnion, choriodecidual and placenta from all gestational categories. Activin A concentrations were, overall, higher than those of inhibin A or follistatin, particularly in the amnion, while inhibin A and follistatin concentrations tended to be similar to each other. Activin A levels in the membranes were not significantly altered with labour or at term or preterm (Fig. 1). However, a significant increase ($P<0.01$) was detected in placental activin A concentrations with term labour, while in the PTD tissues concentrations were significantly lower than those delivered spontaneously at term ($P<0.05$). Inhibin concentrations also increased with term (but not preterm) labour in the placenta ($P<0.05$), while they decreased with labour in amnion and choriodecidual ($P<0.05$) (Fig. 2). Choriodecidual inhibin concentrations were also lower in the preterm tissues compared with the TNL group ($P<0.01$), but in the PTD amnion they were higher than TSL tissues ($P<0.01$). Follistatin concentrations were relatively constant across all tissues, with the exception of a small but significant decline ($P<0.05$) in placental tissues in the PTD group (Fig. 3); no significant changes in follistatin concentrations were observed in amnion or choriodecidual tissues.

Further analyses of the data on the PTD tissues were performed subdividing the tissues according to presence of intrauterine infection, spontaneous rupture of the membranes, tocolysis, Caesarean delivery, or twin

Figure 1 Concentrations of activin A in amnion, choriodecidual and placental tissues delivered at term before labour (TNL; $n=15$), after spontaneous labour (TSL; $n=15$) or preterm (PTD; $n=31$). The black boxes represent the 10th–90th percentile, while the range is indicated by upper and lower error bars. The median is indicated by the white line. * $P<0.05$; ** $P<0.01$ (Mann–Whitney U-test).

pregnancy. No significant differences were found in the concentrations of activin A, inhibin A or follistatin between any of these sub-groups in any of the tissues. In the group with evidence of infection concentrations of all three hormones tended to be lower than in non-infected tissues but this difference did not reach statistical significance (Table 2).

Activin A, inhibin A and follistatin concentrations were significantly correlated between amnion and choriodecidual tissues from all three gestational groups (Table 3). No significant correlations were observed in the concentrations of any analyte between choriodecidual and

Figure 2 Concentrations of inhibin A in amnion, choriodecidual and placental tissues delivered at term before labour (TNL; $n=15$), after spontaneous labour (TSL; $n=15$) or preterm (PTD; $n=31$). The black boxes represent the 10th–90th percentile, while the range is indicated by upper and lower error bars. The median is indicated by the white line. * $P<0.05$; ** $P<0.01$ (Mann–Whitney U-test).

Figure 3 Concentrations of follistatin in amnion, choriondecidua and placental tissues delivered at term before labour (TNL; $n=15$), after spontaneous labour (TSL; $n=15$) or preterm (PTD; $n=31$). The black boxes represent the 10th–90th percentile, while the range is indicated by upper and lower error bars. The median is indicated by the white line. * $P<0.05$ (Mann–Whitney U-test).

placental tissues, or amnion and placental tissues. Concentrations of activin A and inhibin A were significantly associated with each other in all of the term amnion and choriondecidua tissues, and also in the amnion preterm tissues (Table 4). Weaker associations were detected in the placental tissues. Activin A and follistatin concentrations were significantly correlated in all membrane samples, and also in the TSL and PTD placental samples (Table 4). Neither activin A, inhibin A, nor follistatin concentrations were significantly correlated with gestational age in any of the tissues from any of the three groups (analysed either individually or collectively). The concentrations of activin A in the tissue homogenates relative to those of inhibin A and follistatin were compared to assess potential changes in the amounts of activin A bioactivity present in the tissues. In general, increasing ratios of activin:inhibin or activin:follistatin were observed in the membranes with labour at term and preterm (Fig. 4), although a significantly lower activin:inhibin ratio was observed in preterm amnion tissues relative to term laboured tissues. Of the other changes, only the activin:inhibin ratio in amnion

Table 3 Correlations (r_s) between amnion–choriondecidua, choriondecidua–placental, and amnion–placental concentrations of activin A, inhibin A and follistatin

		Correlation (r_s)		
		AM/CD	CD/PL	AM/PL
TNL	Activin	0.732**	0.018 ^{NS}	0.086 ^{NS}
	Inhibin	0.564*	0.418 ^{NS}	0.161 ^{NS}
	Follistatin	0.775***	0.243 ^{NS}	0.146 ^{NS}
TSL	Activin	0.825***	0.011 ^{NS}	0.268 ^{NS}
	Inhibin	0.821***	0.436 ^{NS}	0.268 ^{NS}
	Follistatin	0.261 ^{NS}	0.125 ^{NS}	0.136 ^{NS}
PTD	Activin	0.766***	0.202 ^{NS}	0.375*
	Inhibin	0.535***	0.054 ^{NS}	0.127 ^{NS}
	Follistatin	0.691***	0.007 ^{NS}	0.028 ^{NS}

AM, amnion; CD, choriondecidua; PL, placenta.

* $P<0.05$; ** $P<0.01$; *** $P<0.005$; NS, not significant ($P>0.05$).

and choriondecidua tissues and the villous placental activin:follistatin ratio with term labour proved to be significant.

Conclusions

In the present study we have investigated labour-associated changes in intrauterine biosynthesis of activin A, inhibin A and follistatin by determining concentrations of these hormones in tissues snap frozen shortly after delivery. While acknowledging that this approach does not allow synthesis to be distinguished from either secretion or accumulation, it does allow an accurate assessment to be made of local concentrations of the hormones in question which in turn will reflect components of all of these processes.

Our findings indicate that activin A is more abundant than inhibin A in gestational tissues. This is consistent with indirect evidence from immunohistochemical studies of inhibin subunit localisation and expression (Minami *et al.* 1992, Petraglia *et al.* 1993b). Follistatin has been previously localised to the extraplacental membranes and placental syncytiotrophoblast at term by immunofluorescence (Petraglia *et al.* 1994a), and has been identified in placental

Table 2 Concentrations of activin A, inhibin A and follistatin in preterm tissues categorised as with (+) and without (–) evidence of intrauterine infection. Data shown as pg/mg protein, median (interquartile range)

	Infection status	<i>n</i>	Amnion	Choriondecidua	Placenta
Activin	–	24	3277 (1865–4986)	2893 (2364–6845)	2063 (1640–2741)
	+	6	2017 (1935–2216)	2004 (1634–4184)	1643 (970–2433)
Inhibin	–	24	233 (124–399)	343 (193–551)	592 (450–834)
	+	6	211 (197–228)	196 (146–647)	655 (291–684)
Follistatin	–	24	368 (169–437)	415 (268–645)	375 (282–550)
	+	6	229 (107–310)	186 (146–544)	493 (392–554)

Table 4 Correlations between activin and either inhibin or follistatin concentrations in amnion, choriodecidua and placental tissue extracts

		Correlation (<i>r</i> _s)		
Variable		TNL	TSL	PTD
Amnion	Inhibin	0.871***	0.779***	0.376*
	Follistatin	0.829***	0.529*	0.421*
Choriodecidua	Inhibin	0.871***	0.818***	0.224 ^{NS}
	Follistatin	0.568*	0.771***	0.613***
Placenta	Inhibin	0.618*	0.254 ^{NS}	0.539***
	Follistatin	0.218 ^{NS}	0.571*	0.488**

P*<0.05; *P*<0.01; ****P*<0.005; NS, not significant.

homogenates by chromatography and immunoassay (de Kretser *et al.* 1994), but its presence in intrauterine tissues has not been previously quantified. While our data indicates that concentrations of follistatin in gestational tissues are lower than those of activin A, it should be noted that our follistatin ELISA is relatively specific for follistatin 288, cross-reacting only ~10% with the 315 isoform. The study of de Kretser *et al.* (1994) reported the presence of three main peaks of follistatin immuno-reactivity in term placental homogenates, consistent with molecular weights of 31, 35 and 38 kDa which presumably correspond to follistatins 288, 303 and 315. Corresponding data on follistatin isoforms in the extraplacental membranes is not available. Hence our results probably underestimate the amount of follistatin present in the tissues, at least in the placental samples, although the extent to which this occurs cannot be accurately estimated.

Our findings suggest that placental activin and inhibin production increase modestly with labour at term, and hence are consistent with the finding of elevated activin concentrations in maternal serum after term labour (Petraglia *et al.* 1994b). However, our observation of declining inhibin A concentrations and unchanging activin concentrations with term labour in amnion and choriodecidua would argue against these tissues being the source of the increase in activin A and inhibin A concentrations in amniotic fluid reported recently (Petraglia *et al.* 1997). Interestingly, however, we did observe that the activin:inhibin ratio (and to a lesser extent the activin: follistatin ratio) in the membranes increased following term labour. This observation would be consistent with a labour-associated increase in activin bioactivity in the extra-placental membranes, although an accurate assessment of the relative ratios of activin and follistatin would require measurement of all follistatin forms, not just the 288 form measured by the present assay. The reported decline in amniotic fluid follistatin concentrations at term (Evans *et al.* 1998) would also support such an occurrence. Activin A has been reported to stimulate prostaglandin production by an amnion-derived cell line (Petraglia *et al.* 1993b). Although we have been unable to reproduce this

Figure 4 Changes in the ratio of activin:inhibin and activin: follistatin in gestational tissues taken at term before labour (TNL), after labour (TSL) or preterm (PTD). A, amnion; B, choriodecidua; C, placenta. The bars represent the median with the interquartile range indicated by the error bars. Statistical differences were determined by Mann-Whitney U-test.

finding, we have recently demonstrated an effect of activin A on prostaglandin E₂ production by amnion explants (Keelan *et al.* 1999). Effects of activin on placental hormone production have been documented which may impact on the mechanism or timing of labour (Petraglia *et al.* 1989, Florio *et al.* 1995, Song *et al.* 1996). Activin may also play hitherto unrecognised roles in the mechanism of parturition including local effects on cytokine function (Yu *et al.* 1998, Keelan *et al.* 1999).

The strong correlations in the concentrations of activin A, inhibin A and follistatin between amnion and chorio-decidual tissues from the same pregnancies is consistent with their accumulation from adjacent membranes. Alternatively, the concentrations of activin A, inhibin A and follistatin measured in the tissue samples may principally reflect synthesis *in situ*, with amnion and choriodecidual production (but not placental production) subject to similar regulatory influences *in vivo*. This interpretation is supported by the strong correlation between activin A and either inhibin A or follistatin in all three gestational tissues, which suggest that the synthesis of these three polypeptides is regulated in a similar fashion in response to regulatory signals in these tissues. Indeed, activin itself may be the common link, since inhibin and follistatin synthesis and secretion have been shown to be regulated by activin in a number of tissues (La Polt *et al.* 1989, Bilezikjian *et al.* 1993, Song *et al.* 1996).

Petraglia and co-workers (1995, 1997) have described elevated free activin A concentrations in amniotic fluid and maternal plasma of women who delivered preterm. Unexpectedly, we did not observe an increase in activin A or inhibin A concentrations in any of the preterm tissues relative to those at term. This also contrasts with the findings of Petraglia *et al.* (1997) who noted increased activin A mRNA abundance in amnion and chorion tissues from pregnancies delivered preterm. However, these findings were based on a semi-quantitative RT-PCR analysis of only five–seven samples, and it is possible that increased mRNA expression may not be reflected in increased protein synthesis. The present findings are consistent with the hypothesis that placental activin A and inhibin A production is not associated with any of the pathological processes that lead to preterm labour and delivery, and our measurements simply reflect a very modest gestational age-related increase in their expression (despite the lack of significant correlation with gestational age). In this respect, the lack of suitable control material for the preterm tissues is a significant, but unavoidable, limitation of this study. An alternative explanation for our observations is that glucocorticoids, administered to all pregnancies in this study that delivered preterm, might have suppressed intrauterine production of inhibin A and activin A in the preterm tissues to levels similar to those at term. Activin A biosynthesis and production by bone marrow stromal cells (Shao *et al.* 1998) and monocytes (Yu *et al.* 1996) has been shown to be inhibited by glucocorticoids, although similar studies have not been reported in placental tissues. This explanation seems unlikely, however, as we have measured cytokine concentrations in the preterm amnion and choriodecidual tissue homogenates and found them to be markedly elevated compared with tissues delivered at term (Keelan *et al.* 1999), indicating that inflammatory reactions in the preterm amnion and choriodecidual tissues were not markedly suppressed by antenatal glucocorticoid therapy.

Additionally, we have evidence that circulating maternal activin A concentrations are not suppressed after glucocorticoid treatment (Coleman *et al.*, submitted). We also failed to find a significant increase in activin A or inhibin A concentrations in the membranes of the pregnancies with evidence of infection. This was unexpected since we have recently established that inflammatory cytokines stimulate activin production by amnion, decidual and placental cells *in vitro* (Keelan *et al.* 1998). However, interpretation of these data are compromised by our ability to accurately diagnose those pregnancies with intrauterine infection. Our categorisation of these pregnancies was based on the presence of two or more of several clinical indicators of intrauterine infection, and histological examination was available only on a small number of these placentae. Hence, further studies are required to assess the effects of intrauterine infection on placental activin/inhibin expression *in vivo*.

In conclusion, we have measured activin A, inhibin A and follistatin concentrations in gestational tissues and observed an increase in placental activin A and inhibin A concentrations with term labour. Inhibin A concentrations in the extra placental membranes appeared to decline with term labour, and the resulting increase in the ratio of activin to inhibin may be reflected in a local increase in activin A bioactivity with labour. Concentrations of activin A, inhibin A and follistatin were not elevated in the tissues from preterm deliveries compared with term deliveries, and the data do not support an association between increased activin A production and preterm labour and delivery.

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