Inhibins and activins in human ovulation, conception and pregnancy

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The activins and inhibins are glycoproteins that belong to the transforming growth factor-β (TGFβ) superfamily and, as such, have diverse effects at many stages during growth and development. Originally identified by their effects on follicle stimulating hormone in males and females, the recent development of specific and sensitive assays for this group of polypeptides has permitted the elucidation of their role in ‘fine-tuning’ the hypothalamic–pituitary–gonadal axis. This review article focuses on the roles of inhibin and activin in female reproductive physiology with reference to possible future clinical applications in the investigation of infertility and abnormal pregnancy.

Key words: activin/conception/inhibin/ovulation/pregnancy

Introduction

The identification, purification and subsequent cloning of the members of the inhibin–activin superfamily (Figure 1) and the subsequent development of sensitive and highly specific two-site enzyme-linked immunoassays for these polypeptide hormones has provided tentative answers to many of the outstanding questions concerning the regulation of the hypothalamic–pituitary–gonadal axis. The existence of a non-steroidal regulator of follicle stimulating hormone (FSH) was first predicted by Mottram and Kramer (1923), who demonstrated that the destruction of seminiferous tubules in rats by X-radiation of the testis caused hypertrophy of the anterior pituitary. Subsequently, McCullagh (1932) reported the observation that administration of a water-soluble extract of bovine testis could block this pituitary hypertrophy and he coined the term ‘inhibin’ for this putative factor. The development of radioimmunoassays in the 1970s permitted the measurement of FSH and it was demonstrated that FSH could be selectively blocked by testicular and follicular fluids (Franchimont et al., 1972; Setchell and Jacks, 1974; Keogh et al., 1976).

Inhibin was isolated from bovine follicular fluid in 1985 (Robertson et al., 1985) and soon afterwards from porcine, ovine and human follicular fluid (Ling et al., 1985). The discovery of a structurally related but functionally antagonistic protein, characterized as activin, which stimulated the production and secretion of FSH, followed in 1986 (Vale et al., 1986; Ying, 1988).

Inhibin and activin were thought to act solely on the pituitary gland in a classical endocrine feedback loop; however, mRNA for these factors have since been described in a number of other tissues (Meunier et al., 1988) such as placenta, adrenal, pituitary, bone marrow, kidney, spinal cord and brain, suggesting more diverse biological functions.

Structure of inhibin and activin

Inhibins and activins are glycoproteins that belong to the transforming growth factor-β (TGFβ) superfamily (Kingsley, 1994). Inhibins are heterodimers consisting of disulphide-linked α (mol. wt 18 kDa) and β (mol. wt 14 kDa) subunits: inhibin A (α-βA) and inhibin B (α-βB) show considerable homology of amino acid sequence and,

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whereas the alpha subunits in the two different forms of inhibin are identical, there is 70% homology between the \( \beta_A \) and \( \beta_B \) subunit amino acid sequences and there is 30% homology between the sequences for the \( \alpha \) and \( \beta \) subunits. Only the dimeric forms of inhibin are bioactive, although the \( \alpha \) subunits circulate in excess as biologically inert monomers. The activins are dimers of \( \beta \) subunits and act as functional antagonists of inhibin to increase FSH release by the pituitary. Activin A is a homodimer of \( \beta_A \) subunits and is identical to erythroid differentiation factor (EDF) isolated from a human leukaemic cell line (Eto et al., 1987). Activin AB is a \( \beta_A - \beta_B \) dimer and the third form, activin B (\( \beta_B - \beta_B \)), has been isolated from human follicular fluid (Figure 1). The inhibin/activin dimers are members of the TGF-\( \beta \) family and share the \( \beta \) subunit structure. TGF-\( \beta \) is the prototype of factors involved in a wide range of cellular processes including apoptosis, cell death, proliferation and differentiation (Massague, 1990). Other members of this family include Müllerian inhibiting substance, bone morphogenic proteins and the amphibian protein VG1 which plays an important role during embryogenesis.

Receptors and binding proteins

Two classes of activin receptors have been identified: type II (actRII), which binds activin with high affinity only in the presence of type I (actRI), which acts as a signalling peptide (Mathews and Vale, 1991).

Both the type I and type II receptors act via serine/threonine kinase pathways and were the first such receptors to be identified in vertebrates. The activin II receptor will bind inhibin, but with a much lower affinity than activin (Mathews, 1994), raising the possibility that some actions of inhibin are executed via heteromeric receptor complexes. An alternative hypothesis is that a separate inhibin receptor or inhibin accessory protein exists that mediates an inhibin-specific signal. Supporting this hypothesis is the identification of inhibin-specific binding sites on ovarian granulosa cells and testicular Leydig cells (Woodruff et al., 1993; Woodruff and Mather, 1995) and the identification of an inhibin-specific protein complex in a haematopoietic cell line (K562) (Lebrun and Vale, 1997).

Gonadal tumours that develop in mice which are genetically deficient in the inhibin \( \alpha \) subunit ('inhibin knock-out mice') have been shown to bind inhibin specifically and four distinct inhibin-binding proteins were purified by affinity chromatography from these tumours (Draper et al., 1998).

Follistatin is a structurally unrelated monomeric protein which suppresses pituitary FSH release (Robertson et al., 1987; Ueno et al., 1987; Michel et al., 1993) and thus functions as a weak agonist of inhibin. The molecule is encoded by a single gene and alternative splicing of gene transcripts yields several size variants. In addition to suppressing pituitary FSH, follistatin binds to activin and, to a lesser extent, to inhibin through their common \( \beta \) subunit (Muttukrishna et al., 1995). This represents follistatin’s major functional role as it acts to neutralize activin bioactivity in the circulation since the affinity of follistatin for activin is similar to that of activin and its receptor. Activins and inhibin are also bound by \( \alpha_2 \)-macroglobulin, which shows a lower affinity for them than follistatin does, but which is found in much higher concentrations in serum (Krummen et al., 1993).
Assays for inhibins and activins

The first assays for inhibin were bioassays using dispersed anterior pituitary cells (Vale et al., 1972). Inhibin concentrations could be estimated in vivo by assessing the suppression of FSH secretion into culture media. (Eddie et al., 1979). However, the interpretation of these assays was complicated by the interaction of inhibin with activin, binding proteins and other FSH modulators in vivo.

Radioimmunoassays, such as the ‘Monash’ assay developed by McLachan and co-workers (McLachan et al., 1986a, 1987a; Schneyer et al., 1990) in Australia, were subsequently developed and provided a wealth of insight into reproductive physiology. However, the ‘Monash’ assay, which used a polyclonal antibody raised against 31 kDa bovine inhibin with epitopes for the antibody on the inhibin α subunit, is unable to discriminate between dimeric bioactive inhibin forms and various forms of free α subunit which circulate in 20-fold excess. Several groups prepared monoclonal and polyclonal antibodies with the intention of producing two-site immunoassays to measure specifically the bioactive dimeric forms of inhibin (Illingworth et al., 1991; Baly et al., 1993; Poncet and Franchimont, 1994). However, due to the high degree of structural conservation of inhibin between species, it is a poor immunogen and the antibodies raised had limited affinity.

More recently, a panel of monoclonal antibodies to synthetic peptide immunogens were used to construct ultra-sensitive enzyme-linked immunosorbent assays (ELISA) for dimeric inhibin A, inhibin B, pro α-C, activin A and activin AB (Groome et al., 1994, 1995, 1996; Knight and Muttukrishna 1994; Evans et al., 1997). The inhibin A, inhibin B, activin A and activin AB assays all make use of a sample pre-treatment process with hydrogen peroxide (Knight and Muttukrishna, 1994) which oxidizes the methionine residues in the β subunits and significantly increases the sensitivity of the assays. An additional specificity-enhancing step involves heating samples for these assays with a sodium dodecyl sulphate solution, which irreversibly disrupts activin/follistatin complexes and, in the inhibin B assay, removes the effect of heterophil antibodies.

A very high specificity and sensitivity is required for measuring concentrations of the various members of the inhibin/activin superfamily in serum where physiological concentrations may be detected at concentrations as low as 5 pg/ml. The Groome ELISAs, which are now available commercially (Serotec, Kidlington, UK), provide precise and replicable results for use in clinical and physiological research.

Inhibin and activin in the ovarian cycle

Regular, cyclical mono-ovulation in women is achieved by a complex interaction of hormonal signals which prevent the ‘default to atresia’, which is the fate of 99.98% of the approximately 2 000 000 primordial follicles that exist in the ovaries of the new-born female infant (Gosden, 1985). Pre-antral stages of follicular growth occur independently of gonadotrophic stimulation (Baker and Scrimgeour, 1981). However, antrum formation requires stimulation by FSH, acting via its receptor in the granulosa cell surface membrane. From puberty, cyclic increases in pituitary FSH secretion rescue a cohort of follicles from atresia according to the ‘threshold’ concept (Brown, 1987). Although multiple follicles are recruited to begin pre-ovulatory development, as the FSH concentration rises at the beginning of each cycle, usually only one survives to become dominant — the follicle whose granulosa cells are most responsive to FSH. Development of the dominant follicle is characterized by the secretion of increasingly large amounts of oestradiol and inhibin A into the circulation. There is evidence to suggest that the maintenance of dominance is effected by intra-ovarian paracrine signalling (Hillier, 1981) with inhibins and activins acting as important paracrine messengers (Hillier, 1991). Inhibin B rises from early in the follicular phase to reach a peak coincident with the onset of the mid-follicular phase decline in FSH concentrations and then declines during the luteal phase apart from a peri-ovulatory peak which may represent release of follicular inhibin B from the rupturing follicle into the circulation (Groome et al., 1996) (Figure 2). By contrast, inhibin A concentrations are low in the early follicular phase, show a small mid-follicular phase peak, rise rapidly with ovulation and are maximal during the mid-luteal phase. Prostaglandin E2 has been shown to be an important inducer of inhibin A in granulosa cells in culture and it has been suggested that a local action of PGE2 may partially explain the increase in circulating inhibin A observed during the midluteal phase of the cycle (Erämaa and Ritvos 1996). During the luteo–follicular transition, inhibin B concentrations rise rapidly to their mid-follicular peak whereas inhibin A concentrations fall synchronously with oestradiol and progesterone to reach a nadir at the time of the intercycle FSH peak. The different patterns of circulating inhibin A and B during the two phases of the ovarian cycle are strong evidence for their playing different physiological roles during follicular recruitment, maturation and ovulation (Groome et al., 1996). The hypothesis that inhibin B may play an important paracrine role in developing follicles and a significant regulatory role with respect to the suppression of FSH is...
supported by measurements of inhibin A, B and activin A in the follicular fluid of follicles of a range of sizes in regularly cycling women (Magoffin and Jakimiuk, 1997). These investigators found that emerging dominant follicles that were destined to ovulate, i.e. had an androstenedione:oestradiol ratio ≤ 4, had higher concentrations of inhibin A than follicles with an androstenedione:oestradiol ratio > 4. Inhibin B concentrations were higher than inhibin A concentrations in all follicles with a peak of 409 ± 9.6 ng/ml in 13 mm follicles.

During the menstrual cycle, serum activin A concentrations vary in a biphasic manner with highest concentrations at mid-cycle and nadirs in the mid-follicular and mid-luteal phases.

**Inhibins and activins in ovulation induction cycles**

Gonadotrophin releasing hormone (GnRH) analogue (pituitary desensitization) treatment cycles are widely used in assisted reproductive therapies such as gamete intra-Fallopian transfer (GIFT) and in-vitro fertilization (IVF). Pituitary desensitization produces a marked suppression of inhibin A with a more modest suppression of inhibin B and no significant effect on inhibin pro α-C or activin A when compared to normal early follicular phase concentrations. (Figure 3) (Lockwood et al., 1996a). Stimulation with FSH produces large increases in inhibin A and B and pro α-C with highly significant correlations being seen between the number of large follicles developing and inhibin A, pro α-C and oestradiol concentrations. The rise in inhibin during gonadotrophin stimulation corresponds to earlier observations during cycles stimulated with clomiphene citrate–human menopausal gonadotrophin (McLachlan et al., 1987b; Tsuchiya et al., 1989) and without GnRH analogue pre-treatment (Hughes et al., 1990), and adds weight to the suggestion that measurement of inhibins might indicate the level of follicular recruitment and maturation during ovarian hyperstimulation cycles and possibly be a means of predicting and monitoring ovarian hyperstimulation syndrome (OHSS).
Studies of inhibin B in hyperstimulated cycles have shown that very high concentrations (>1000 pg/ml) are generated in the presence of multiple follicles (Lockwood et al., 1996a) whereas the mid-follicular phase rise in inhibin B in spontaneous cycles originates from the few pre-dominant follicles. In ovulation induction cycles, the multiple co-dominant follicles are presumably responsible for the high concentrations of inhibin B found and this observation is consistent with studies showing that small follicles express more βB mRNA (Roberts et al., 1993).

Inhibins and activins in polycystic ovary syndrome

Recent advances have been made in our understanding of inhibin physiology by studying naturally occurring dysovular states such as polycystic ovary syndrome (PCOS) and Kallmann’s syndrome. There have been conflicting reports in the literature concerning the inhibin status of women with PCOS, one of the most common endocrine disorders affecting ovulation (Polsson et al., 1988; Clayton et al., 1992). The aetiology of PCOS is unclear, but its effective treatment by both anti-oestrogens and by exogenous FSH suggests that a primary disorder of FSH regulation may be central. To investigate a possible role of inhibin B in the pathophysiology of PCOS, serum inhibin B concentrations were measured in 10 women with PCOS on cycle day 5 of a spontaneous or progestogen-provoked bleed and compared with concentrations on cycle day 5 of 10 women with regular, ovulatory cycles. The mean serum inhibin B concentrations in the PCOS patients were significantly higher (Lockwood et al., 1998a) compared with normal controls, and this finding is consistent with the exquisite sensitivity to exogenous FSH seen in PCOS patients undergoing fertility treatment (Figure 4). Serial blood sampling on cycle day 5 of women with clomiphene-resistant PCOS and normal controls revealed that in ovular women there is a distinct periodicity in inhibin B concentrations with a clear peak detectable every 60–70 min (Lockwood et al., 1998a) whereas in women with ovulatory dysfunction due to PCOS no such pattern of regular pulsatility was seen (Figure 5).

The function of inhibin B pulses in the mid follicular phase of the normal cycle remains to be elucidated, but the absence of the normal pulsatile pattern in women with PCOS, in conjunction with high basal concentrations of inhibin B arising from the multiple small follicles characteristic of the PCOS ovary, appears to reinforce the development of a large cohort of small, developmentally arrested and ultimately atretic follicles in these patients. If PCOS is interpreted as a disease of inappropriately low FSH secretion (Yen et al., 1970) then we can explain the characteristic hormone profile of low/normal FSH in conjunction with elevated LH as a response to elevated inhibin B secretion. This hypothesis is supported by pituitary cell culture studies in sheep (Muttukrishna and Knight, 1991) and rats (Dahl et al., 1992) which demonstrate that FSH but not LH secretion is suppressed in vitro by inhibin. The ‘elevated inhibin B’ hypothesis therefore, whether it represents an initiation of, or a response to, pituitary–ovarian dysfunction, offers a unifying neuroendocrine explanation for the aetiology of PCOS.

Hypogonadotrophic states such as Kallmann’s syndrome provide an opportunity to explore the relationship between circulating concentrations of inhibins, activins, gonadotrophins and steroids during stimulated folliculogenesis. We have previously reported that stimulation with recombinant FSH (with no LH activity) results in the development of ultrasonographically normal but functionally abnormal follicles with very low oestradiol and inhibin concentrations (Lockwood et al., 1996b). In hypophysectomized rats it has similarly been demonstrated that FSH-regulated inhibin subunit mRNA expression requires the presence of LH (Aloi et al., 1995).

Perimenopause

An established endocrine finding associated with ageing is the rise in circulating FSH concentrations. Pituitary FSH is co-regulated by oestradiol and inhibins. During the early stages of ageing, changes in oestradiol that could fully account for the rise in FSH have not been consistently observed (Metcalf and Livesey, 1985; Lee et al., 1988). A large cross-sectional study of the menopausal transition...
using the Monash assay found that immunoreactive inhibin decreased with advancing age and was positively correlated with oestradiol and negatively correlated with serum FSH (Burger, et al., 1995). A recent study has shown that early follicular phase concentrations of inhibin B were significantly lower in older women with raised FSH \( (n = 7) \) than in control younger women \( (n = 7) \) with normal FSH (Klein et al., 1996). Our studies using older women with normal and raised FSH show that both inhibin A (predominantly originating from the dominant follicle) and inhibin B (from the small follicles) are lower during the cycle in women with raised FSH (day 3 FSH >8 mIU/ml; \( n = 4 \)) and normal FSH (day 3 FSH <8 mIU/ml; \( n = 10 \)) Muttukrishna et al., unpublished). We could explain the rise in FSH without a change in oestradiol as being due to the decline in the negative feedback effect of inhibins on the pituitary in perimenopausal women. Collectively these studies suggest that inhibin A (mid–late follicular phase) and inhibin B (early follicular) measurements in the follicular phase of the menstrual cycle could be valuable in predicting the menopausal transition.

Recently a new role for inhibin B as a marker of ovarian reserve has been suggested (Lockwood et al., 1997a,b; Seifer et al., 1997) drawing on the observation in vitro that \( \beta_B \) mRNA is predominant in small antral follicles, which are recognized as being depleted in women with poor ovarian reserve. Conventionally day 3 FSH concentrations have been used as a predictor of ovarian reserve in perimenopausal and infertile women. However, day 3 serum FSH in perimenopause is not consistent between cycles in some women although we have found a high degree of consistency of inhibin B concentrations between cycles in women with significant inter-cycle variation in basal FSH (Lockwood et al., 1998b).

We have recently demonstrated that in normal controls and fertility patients with tubal or male factor infertility there is a strong inverse correlation between early follicular phase FSH and inhibin B, but this correlation disappears when women with unexplained infertility or unexpectedly poor response to gonadotrophin stimulation are investigated. (Lockwood et al., 1997a) (Figure 6).

**Inhibin and activin during pregnancy**

Serum concentrations of immunoreactive (ir) inhibin have been reported to be higher during various stages of preg-
nancy compared with non-pregnant subjects (McLachlan et al., 1987; Abe et al., 1990; Tabie et al., 1991; Yohkaichiy et al., 1991; Baird and Smith, 1993; Tovanabutra et al., 1993). In all of these previous studies ir-inhibin was measured using radioimmunoassays which cross-react extensively with different inhibin α subunit forms. Consequently, the contributions of the biologically active dimeric inhibin forms were unknown. Development of specific and sensitive assays for dimeric inhibins has facilitated the measurement of these proteins throughout human pregnancy (Muttukrishna et al., 1995). In human pregnancy, inhibin A is the major circulating form of inhibin with concentrations of inhibin B near the detection limit of the assay (S.Muttukrishna, unpublished observation). As shown in Figure 7, serum inhibin A concentrations steadily decrease from 8 weeks up to 16 weeks gestation. Concentrations remained low throughout the second trimester and then increased ~5-fold during the third trimester and reached a maximum concentration at 36 weeks gestation. Serial changes in the concentrations of inhibin A and inhibin B during the establishment of pregnancy have been reported by Illingworth et al. (1996). In spontaneous singleton pregnancies, inhibin A concentrations start rising markedly from 5 weeks to reach a peak concentration at 8 weeks gestation. After 8 weeks inhibin A concentrations start to decline up to 11 weeks, consistent with the pattern reported in the cross-sectional study (Muttukrishna et al., 1995). Inhibin B concentrations did not rise in early pregnancy and concentrations were near the detection limit of the assay (10 pg/ml).

Previous studies using the Monash radioimmunoassay have reported a major luteal contribution of inhibin A in early pregnancy (McLachlan et al., 1987a; Yohkaichiy et al., 1991).

Immunolocalization studies have shown inhibin and activin subunits (both α and β) in first trimester human fetuses (Harkness and Baird, 1997a) and in-situ hybridization has localized inhibin mRNA αA and βA subunits in a variety of fetal tissues (Harkness and Baird, 1997b). It has been demonstrated that inhibin and activin influence hormonegenesis in cultured placental tissue, with activin increasing GnRH and progesterone production. These results support the hypothesis that the inhibin superfamily may be implicated in the endocrine physiology of pregnancy (Petraglia et al., 1989).

Measurement of activins in circulation has been difficult due to the presence of the high affinity activin binding protein follistatin. Despite these limitations, several groups have reported the measurement of activins in biological fluid using various assay formats including bioassay (Sakai et al., 1992), radioimmunoassay (Robertson et al., 1992), enzyme immunoassay (Wong et al., 1993; Woodruff et al., 1994) and competitive protein binding assay (Demura et al., 1993). However, the measurement of ‘total’ (bound activin + free activin) in circulation was only possible after the development of a sensitive, specific and accurate enzyme immunoassay (Knight et al., 1996). Using this assay circulating concentrations of ‘total’ activin A have been measured throughout pregnancy (Muttukrishna et al., 1995a). By 8 weeks gestation serum activin A concentrations were higher than those during the normal menstrual cycle (Figure 7). Concentrations of activin A did not vary significantly during the remainder of the first and second trimesters. After 24 weeks, serum activin A concentrations rise with a marked increase at term.

The marked increase in inhibin A and activin A near term suggests a role for these proteins in labour. Recent studies in the rat using a ligand binding assay have shown the presence of activin receptors in pregnant myometrium. Clearly, further research has to be carried out to investigate the biological significance of activin A in parturition.

The corpus luteum is now recognized to be the major site of inhibin A production during the luteal phase of the ovarian cycle (Muttukrishna et al., 1994). However, there has been conflicting evidence about the source of inhibin A production during early pregnancy. Trophoblast expresses inhibin α, βA and βB subunit messenger RNA as demonstrated by Northern blot analysis (Baird and Smith, 1993). Immunohistochemistry has demonstrated inhibin subunit
production by the trophoblast, and cell culture studies have shown that placental trophoblast produces ir-inhibin (McLachan et al., 1986). The donor egg model allows for the separation of the endocrine contribution of the feto-placental unit from that of the corpus luteum since donor egg conceptions are brought about in women with ovarian failure or complete ovarian suppression achieved with GnRH agonists. Comparative studies of conceptions on donor egg programmes were in conflict about the source of ir-inhibin as McLachlan et al. (1987b) reported similar concentrations of ir-inhibin in the maternal circulation of pregnant women with functional ovaries and those conceiving with donor eggs, whereas a study by Yohkaichiya et al. (1991) showed decreased concentrations in women pregnant without corporo-luteal function. Our recent study (Muttukrishna et al., 1997a) of the endocrine consequences of first trimester surgical termination of pregnancy suggests that the feto-placental unit is the major source of inhibin A in early pregnancy. A recent study (Birdsall et al., 1997) again used the donor-egg model in conjunction with the specific ELISA for inhibin A and activin A and concluded that the feto-placental unit was the major source of these polypeptides in early pregnancy. In our recent study (Lockwood et al. 1997c), the source of inhibins in early pregnancy is investigated by comparing inhibin and activin A profiles in pregnancies conceived in vitro with and without corpus luteum function. This use of autologous, frozen–thawed embryo pregnancies as the comparator for IVF pregnancies achieved with fresh embryo transfer and spontaneous conceptions was designed to overcome theoretical objections to the donor-egg model.

This study demonstrated that the elevation of circulating concentrations of dimeric inhibin A in early pregnancy is the result of production by the feto-placental unit since comparable concentrations of inhibin A were found in singleton pregnancies arising from spontaneous conceptions and following IVF treatment with both fresh and frozen embryo transfer. In the first case, a single corpus luteum would be expected, in the second case multiple corpora lutea are routinely observed and in pituitary down-regulated frozen embryo replacement cycles no corpus luteum is present (Figure 8). The significantly higher concentrations of inhibin A found in multiple pregnancies is further evidence for this. The very low concentrations of inhibin A found in pregnancies that became missed abortions and the undetectable concentrations seen in ‘biochemical’ pregnancies, notwithstanding their ‘normal’ human chorionic gonadotrophin (HCG) concentrations, is further evidence for this hypothesis and reflects the short half-life of inhibin A in the circulation as previously demonstrated by Muttukrishna et al. (1997a). The significant differences in concentrations of inhibin A in viable, non-viable and multiple IVF pregnancies detectable as early as 13 days following embryo transfer is further evidence as to the source of inhibin A in early pregnancy and suggests that estimation of inhibin A may be helpful in the management of early pregnancy in IVF patients especially in the presence of bleeding or symptoms of OHSS. Early diagnosis of a complicated or poor pregnancy outcome could aid the counselling and management of this group of fertility patients (Clifford et al., 1997).

The functions of inhibin and activin in early pregnancy are proving difficult to elucidate, but it has been postulated that they may play a paracrine role in early pregnancy recognition. Total activin A rises in the late luteal phase as the corpus luteum is involuting and acts to inhibit basal and HCG-stimulated progesterone production by luteal and granulosa–lutein cells. Activin thus has the potential to inhibit the steroidogenesis of the corpus luteum and blocks its actions. If non-pregnant women in luteal phase are given
exogenous HCG at concentrations which mimic those of early pregnancy, inhibin A and pro-αC concentrations rise and activin A concentrations are suppressed whereas they normally rise late in the luteal phase. In a non-conception cycle, activin within the ovary may be acting to extinguish the function of the corpus luteum prior to menstruation, but activin’s action is suppressed in early pregnancy by HCG and thus corporo-luteal function can continue and the pregnancy be maintained.

Inhibin in abnormal pregnancy

Pre-eclampsia

Pre-eclampsia is a placental disease of unknown cause, the onset and progress of which is unpredictable (Redman, 1991). Inhibin A and activin A concentrations are significantly raised in pre-eclampsia (defined as sustained diastolic pressure $\geq 90$ mm Hg from previously lower concentrations and sustained proteinuria $\geq 0.3$ mg/24 h) compared to gestational age matched controls. All patients reported in this study developed pre-eclampsia at ~30 weeks gestation (Muttukrishna et al. 1997b). As shown in Figure 9, concentrations of inhibin A and activin A in pre-eclampsia were not overlapping between the controls and cases (with a ‘cut off’ value of 1 ng/ml for inhibin A and 10 ng/ml for activin A). Studies carried out in early pregnancy (15–18 weeks gestation) have shown a significant rise (~60%) in inhibin A concentrations in pregnant women who developed pre eclampsia later in pregnancy compared to normal pregnant women (Muttukrishna et al., 1997c). Based on this observation, we could speculate that women with raised inhibin A concentrations at ~16 weeks gestation (in a pregnancy unaffected with Down’s syndrome) are at a higher risk of developing pre-eclampsia. However, further studies have to be carried out with larger number of cases and controls to define a ‘cut off’ concentration.

Inhibin in screening for Down’s syndrome

Antenatal screening for causes of severe handicap, such as trisomy 21, has become an established part of obstetric practice. Although biophysical markers such as detection of increased nuchal fold thickness using ultrasound (Szabo and Gellen, 1990; Nicolaides et al., 1994) have been adopted in some centres, serum testing is still the mainstay of antenatal screening for this condition. Early work focused on the finding of abnormally low concentrations of alpha-fetoprotein (AFP) in sera collected from women carrying a fetus with Down’s syndrome (Cuckle et al., 1984) but it soon became apparent that other serum markers were also abnormally raised or suppressed in cases of trisomy 21, and that these might usefully be combined into screening tests with acceptable sensitivity and specificity.

Three studies using the inhibin α subunit-directed assays (Van Lith et al., 1992; Spencer et al., 1993; Cuckle et al., 1994) and five studies using the dimeric inhibin A assays have shown that measuring maternal serum inhibin A in second trimester would contribute to the existing screening test (Canick et al., 1994; Cuckle et al., 1995; Aitken et al., 1996; Wald et al. 1996a,b; Wallace et al., 1996). Down’s screening results were 77% predictive if inhibin A was added as a fourth marker to the existing triple test (AFP, UE3, βHCG and age) from 15–18 weeks gestation. An advantage of the use of inhibin A is the very small change in average inhibin A from 15 to 18 weeks gestation. The inclusion of inhibin A has significantly improved the predictive value of the existing screening test. Concentrations of inhibin A in amniotic fluid collected from normal preg-
nancies and those affected by Down’s syndrome have also been investigated (Riley et al., 1997). Concentrations were significantly lower in Down’s pregnancies, suggesting a possible origin for inhibin A from the fetal membranes in the second trimester and possible clinical efficacy as a rapid test for the presence of a Down’s fetus by assay of inhibin species in amniotic fluid.

Serum screening before 15 weeks of pregnancy has been largely unreliable in this regard, although there are few studies with sufficient power to identify the true potential of a range of markers in combination. Wald et al. (1996a,b) studied a panel of seven markers, including dimeric inhibin A, in samples collected between 8 and 14 completed weeks of gestation, but failed to demonstrate sufficient elevation of inhibin A in affected pregnancies for it to form a useful adjunct to measurement of free βHCG and PAPP-A in the first trimester. Similarly poor discrimination between affected and normal pregnancies was also shown in a second large series (Noble et al., 1996), although conversely, Wallace et al. (1995a,b) found a more substantial elevation in inhibin A in the first trimester of trisomy 21 pregnancies in a smaller series of cases. However, concentrations of inhibin A are closely correlated with free βHCG in early pregnancy, suggesting that little would be gained by assay of both markers in a screening panel for trisomy 21 in the first trimester (Noble et al., 1996). A clinically useful first trimester serum screen for Down’s syndrome remains elusive, and it seems unlikely that measurement of inhibin will be useful in this application. Nevertheless, screening for Down’s syndrome has been significantly improved by the application of inhibin A assay in the second trimester, illustrating the potential usefulness for inhibin measurements in routine clinical practice.

In summary, rapidly accumulating evidence from diverse areas of research in reproductive physiology suggests that serum concentrations of the inhibins and activin A could be used as a diagnostic tool in the investigation of infertility and abnormal pregnancy. However, further research with large numbers of patients and controls needs to be carried out before application to routine clinical practice can be advocated.

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