Significance of inhibin in reproductive pathophysiology and current clinical applications

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Abstract

The human reproductive process is regulated by complex mechanisms that involve many organs, including the brain, gonads and endocrine system. It has been more than 70 years since the name ‘inhibin’ was used to describe a substance produced in the gonads that negatively regulates pituitary secretion. Inhibin B controls FSH secretion via a negative feedback mechanism. It is a glycoprotein hormone secreted by the Sertoli cells of the testis and granulosa and theca cells of the ovary. Serum inhibin B concentrations are positively related to testicular volume and sperm counts. Current understanding of inhibin physiology and pathology in the human suggests that inhibin B may be of importance as a marker of Sertoli cell function in men with infertility and as a prognostic indicator in women undergoing ovulation induction therapy. Inhibin concentrations are elevated in patients with granulosa cell tumours and in post-menopausal women with mucinous ovarian cancers. Immunoreactivity against the inhibin α-subunit was identified in all cases of adrenal cortical adenoma and carcinoma, and levels are suppressed in the malignant prostate disease. This article discusses the structure, regulation and clinical use of inhibin and other related substances.

Keywords: activins, clinical uses, FSH, inhibins, pathophysiology

Introduction

Inhibin is a hormone whose existence was proposed several decades before it was isolated. Mottram and Cramer (1923) showed that irradiation of rat testes altered the histology of the pituitary glands by inducing ‘castration cells’. Ten years later, McCullagh (1932) observed that aqueous testicular extracts prevented the formation of these ‘castration cells’ in the rat pituitary, hinting at the existence of a non-steroidal gonadal product produced by the testes. This product was eventually isolated from bovine follicular fluid and called ‘inhibin’ (Robertson et al., 1985).

Today, the physiological significance of inhibin is far from clear. Research suggests that inhibin is a main regulator of FSH synthesis and that it exerts paracrine and autocrine effects on gonadal and extragonadal tissues. For many years it was not possible to differentiate the dimeric forms from the free biologically non-active α-subunits and the high molecular weight precursors. Elaboration of the methods, which determine the active dimeric forms of inhibin A and inhibin B, also the proαC fragment of α-subunit, led to accumulation of data about the role of inhibin as a main regulator of the FSH synthesis. Paracrine and autocrine effects on the gonadal and extragonadal tissues also have been characterized with these methods. Even after the advent of the dimeric assay, interpretation of the assay results remains complicated by the fact that inhibin is partly secreted by extragonadal sources.

This article discusses the basic structure, pathophysiology and clinical relevance of inhibin and its related peptides.
Structure of inhibins and related proteins

Inhibins

Inhibin is a disulphide-linked heterodimeric glycoprotein consisting of α- and β-subunits. Inhibin is a member of the transforming growth factor β (TGFβ) superfamily, a group of structurally similar but functionally diverse growth factors, and it shares several structural features with this family. The inhibin α- and β-subunits are synthesized as pro-proteins (pro-α A-NαC and pro-β-β) (Illingworth et al., 1996). There are two forms of β-subunits, βA and βB. The complex αβA is called inhibin A, and the complex αβB is known as inhibin B (Anawalt et al., 1996; Illingworth et al., 1996a; Anderson et al., 1997). The free α-subunits usually do not suppress FSH. Therefore, the bioactivity of the inhibin depends on the formation of a dimeric αβ structure. Only dimeric forms of inhibin are biologically active. The normal range of inhibin B estimated by enzyme immunoassay is variable.

Activins

Activin A is a homodimer of βA-subunits (βAβA), whereas activin B is a homodimer of βB subunits (βBβB) and activin AB is a heterodimer of β-subunits (βAβB) (Ling et al., 1986; Vale et al., 1986). Their biological effect is stimulation of pituitary FSH secretion. It is not known whether they influence the secretion of LH (Ling et al., 1986; Blumenfeld and Ritter, 2001). Inhibin blocks the release of activin-stimulated FSH via non-competitive inhibition (McLachlan et al., 1988).

Inhibin B and the activins are the products of the same precursors. Like inhibins, activins also belong to the TGFβ superfamily (Ying, 1987; Blumenfeld and Ritter, 2001; Chada, 2003). They regulate both Leydig and Sertoli cell functions and germ cell DNA synthesis in the testis (Ying, 1987). These dimers also regulate the theca and granulosa cell functions of the ovary (Hutchinson et al., 1987).

Follistatin

Follistatin is one of the two binding proteins for inhibins and activins (Moore et al., 1994). This group includes three monomeric glycoproteins that are structurally unrelated to inhibins or activins (32, 35 and 39 kD) (DePaolo et al., 1991). Follistatin has a high affinity for activins and neutralizes their biological effects on the hypophysis. The effects of follistatin are similar to those of inhibins (Voutilainen et al., 1991). A more complete analysis of follistatin’s regulation and secretion could be the key to understanding how the inhibins and activins affect the reproductive system and other tissues.

α2-Macroglobulin

α2-Macroglobulin (α2-MG), a 720-kDa glycoprotein consisting of four identical 180-kDa subunits, is the second binding protein for inhibins and activins (Moore, 1994; Wong et al., 2004). It binds to TGFβ and other growth factors and hormones with high affinity. α2-MG is a secretory product of Sertoli but not germ cells. In the systemic circulation, it is produced by hepatocytes (Wong et al., 2004). Research suggests that this macroglobulin does not influence the biological effects of activins and inhibins.

Gonadal and extragonadal secretion of inhibins and related proteins

Inhibin A is found only in circulating blood of women; it is undetectable in men with contemporary methods (Illingworth et al., 1996a). It is the main form of inhibin produced by the dominant ovarian follicle and the corpus luteum. Inhibin B, on the other hand, is released from the smaller follicles and is a dominant form of inhibin during the early follicular phase (Lee, 2001).

Researchers generally agree that the Sertoli cell is the predominant site of inhibin B production in the testes (Andersson et al., 1998a; Young et al., 2000). By using specific monoclonal antibodies against α- and βB-subunits, Andersson et al., established that the germ cells, but not the Sertoli cells, could be immunostained for βB. The Sertoli cells contain only the α-subunits where as the βB-subunits are localized in the pachytene spermatocytes and in the round spermatids (Andersson et al., 1998a). This data invalidated the hypothesis that the Sertoli cells are the only source of inhibin B in adult men. Only biologically inactive α-subunits can be found by the lack of germ cells in the mature testes (Sertoli-cell-only syndrome). It could be concluded that inhibin B is produced by the Sertoli cells but the process depends on the presence of specific germ cells.

Pachytene spermatocytes and round spermatids in the early stages of development may act as major modulators of inhibin synthesis (Andersson et al., 1998a; Andersson, 2001). Further studies could have important clinical significance. If the germ cell type, which influences inhibin B concentration, could be determined, concentrations of inhibin B could be used as a marker of spermatogenesis and also to indicate the level of interruption to spermatogenesis in men with non-obstructive azoospermia. Finally, such information could be useful in the development of male hormonal contraception (Majdic et al., 1997; Andersson, 2000).

The mRNA for both α- and βB-subunits of inhibin have been localized to Leydig cells in adult humans and rats. Leydig cells may produce inhibin and/or activin (Majdic et al., 1997; Andersson et al., 1998a). Recent studies based on methods for inhibin dimeric form measurement failed to show any change in inhibin B concentration after human chorionic gonadotrophin (HCG) administration, but an increase in pro-αC production in the Leydig cells was observed (Kimnigh and Andersson, 2001). Young et al. reported that a month-long course of LH stimulation in patients with hypogonadism did not affect serum inhibin B concentrations, but did increase testosterone concentrations (Young et al., 2000).

In conclusion, measurement of inhibin concentrations may show an interaction between germ cells and Sertoli cells. A disturbance in this interaction could explain why men with Sertoli cell-only syndrome have extremely low concentrations of inhibin despite of the preservation of the Sertoli cells (Andersson et al., 1998a). Obviously, the synthesis of the βB-subunit in reproductive age depends on the spermatogenesis. These conclusions could explain the contradictory results produced by many of the studies that used older methods for
measuring the dimeric and the free α-subunits of the hormone.

Extragonadal inhibin-α, β_A and β_B-subunit expression has been detected in the pituitary gland, spinal cord, brain, kidneys, adrenal glands and placenta (Voutilainen et al., 1991; Voutilainen, 1995; Salmenkivi et al., 2001). The adrenal gland shows strong immunoreactivity against the inhibin α-subunit, especially in the zona fasciculata and zona reticularis (Spencer et al., 1999; Munro et al., 1999) but not in zona glomerulosa or adrenal medulla (Spencer et al., 1992). Salmenkivi et al. (2001) have found significant immunoreactivity of the medulla against β_B and weak activity against β_A in the inner layer of the cortex. The expression of α-subunits in the adrenal gland is much higher than the expression of β-subunits, and there is most likely an excess of free α-subunits in the adrenals (Voutilainen, 1995). Adrenocorticotropic hormone (ACTH), which is secreted from the pituitary gland, stimulates gene expression of α-subunits (Voutilainen et al., 1991; Spencer et al., 1992; Munro et al., 1999). It is not known whether extragonadal secretion influences inhibin blood concentrations. There is a higher concentration of inhibin-like immunoreactivity in the adrenal veins than in the vena cava or peripheral veins, but inhibin cannot be detected in the circulation after bilateral orchidectomy (Anawalt et al., 1996). The paracrine and autocrine role of inhibin in gonadal and extragonadal tissues is still unknown. Recent findings from Cipriano et al. suggest that the lack of inhibin in mice is connected with the development of adrenal, ovarian and testicular tumours (from granulosa or Sertoli cells respectively) (Cipriano et al., 2001). Activin A can be found in the spermatogenic fluid of healthy men but is undetectable after vasectomy (Anderson et al., 1998c).

Follistatin is produced in the bone marrow, ovaries, testes and pituitary (DePaolo et al., 1991; Kagawa et al., 1991). It has also been found in the spermatogenic fluid, where its concentration after vasectomy remains unchanged. These data suggest that prostatic epithelium and seminal vesicles can release follistatin in the spermatogenic fluid (Anderson et al., 1998b).

### Regulation of inhibin secretion

Blood concentrations of inhibin in men of reproductive age fluctuate throughout the day in accordance with testosterone concentrations: concentrations peak in the early morning and bottom out in the evening (Carlson et al., 1999; Kamischke et al., 2001). Concentrations of inhibin also rise and fall over the course of a year similar to LH, FSH and testosterone; blood concentrations are high during June and July and decrease in August (Meriggiola et al., 1996).

The hypothesis that inhibin production by the testis is stimulated by FSH and, in turn, is part of the negative feedback loop regulating FSH secretion in humans was supported by the initial studies that used the new dimeric inhibin assays (Anawalt et al., 1996; Illingworth et al., 1996a; Mahmoud et al., 1996). In 1997, Anderson et al. (1997a) reported an inverse relationship between blood concentrations of inhibin B and FSH in healthy men and a significant positive correlation between inhibin B and sperm concentration in the ejaculate. Many studies suggest that the concentration of inhibin B in men with normal fertility is higher than that of men with impaired spermatogenesis and infertility. The highest concentrations of inhibin B have been detected in a group of highly selected semen donors (Illingworth et al., 1996a). On the other hand, inhibin B concentrations were undetectable in a group of men who had undergone bilateral orchidectomy (Anawalt et al., 1996).

The influence of gonadotrophin-releasing hormone (GnRH) on rat pituitary cell culture that has been treated with inhibin has been investigated. The results of these studies suggest that inhibin affects the pituitary cells in two ways. Low concentrations suppress the synthesis and release of FSH while high concentrations are associated with the degradation of intracellular gonadotrophin (Jenner et al., 1983). Recent studies revealed that inhibin regulates FSH secretion by reducing the amount of activin available at the binding site and also by reducing activin binding with activin type II receptors (Lewis et al., 2000) (see Figure 1). Activin binds to the SMAD family of proteins, which has been shown to increase FSH secretion. Inhibins and follistatin bind to activin receptors on the gonadotroph cells, and therefore prevent the activation of the SMAD signalling pathway (Lebrun and Vale, 1997).

Chemotherapy-induced testicular injury is associated with a decrease in inhibin B concentrations and an increase in FSH concentrations with little change in LH and testosterone concentrations (Anderson, 1997a). These alterations are accompanied by a progressive rise in free α subunit concentrations, which demonstrates the long-stimulating effect of FSH on the Sertoli cells and dissociation between the secretion of monomeric forms (FSH-dependent) and active dimeric forms of inhibin B.

These data have been supported by results of testicular biopsies obtained from infertile men (Bergh and Cajander, 1990). Specifically, immunostaining has shown that the concentration of α-subunits is higher in the biopsies from the infertile men compared with normal men. Of interest is the fact that both Sertoli cells and Leydig cells stained positive for the α-subunits. It is possible that Leydig cells that are influenced by the increased LH stimulation may contribute to the increase in the α -subunits. LH regulates the secretion of inhibin β-subunits (McLachlan et al., 1988). Leydig cells express α- and β-subunits, but they are not the source of inhibin B in adults (Kamischke et al., 2001). Administration of recombinant LH to hypogonadal men or human chorionic gonadotrophin to normal men is unable to raise serum inhibin B concentrations. This suggests that Leydig cells do not contribute to the pool of circulating inhibin B in men (Meachem et al., 2001). However, androgen receptors are expressed in Sertoli cells (Kamischke et al., 2001). It may well be that testosterone and/or LH via testosterone has a modulating effect on the stimulation of inhibin B production in the Sertoli cells.

The dependence of inhibin B secretion on gonadotrophin secretion has been clearly demonstrated by two studies that consisted of men with hypogonadotrophic hypogonadism who received treatment with GnRH. Pulsatile administration of gonadotrophin progressively increased the blood inhibin B concentrations, which were negatively related to FSH secretion. Data also showed that basal concentrations of inhibin before treatment were significantly lower than
Basal concentrations of inhibin B are positively related to the testicular volume and the sperm concentration in the ejaculate. Data suggest that gonadotrophin-independent inhibin B secretion is a marker of seminiferous tubule maturity. GnRH stimulation accelerates sperm maturity, so withdrawal or suppression leads to a decrease in inhibin B concentration, but only to 30% of normal concentrations (Nachtigall et al., 1996). These findings suggest that once peak inhibin B secretion has been induced, full regression does not occur despite complete withdrawal of gonadotrophins, with approximately one half of inhibin B secretion being constitutive (Anawalt et al., 1996; Anderson et al., 1997). Similar results have been reported in men who were treated with supraphysiological doses of testosterone as a prototype of male contraception, both gonadotrophin production and spermatogenesis were suppressed, but only 60% of the men developed azoospermia (Anderson et al., 1997). Direct testicular damage (bilateral orchidectomy or testicular X-ray treatment) markedly reduces inhibin B secretion to the point where concentrations are undetectable in the serum (Wallace et al., 1997b; Petersen et al., 1999a,b).

The production of inhibin B in adults depends on FSH secretion and spermatogenesis. A precise correlation exists between inhibin B and impaired spermatogenesis. Concentrations of inhibin B are usually at their lowest when spermatogenesis has been disrupted in the earliest stage. It is
possible for a man to have Sertoli cell syndrome and normal concentrations of inhibin, but the reasons are unknown (Foresta et al., 1999).

Inhibin is an important modulator of the reproductive function on the endocrine concentration through the regulation of FSH biosynthesis. Hayes et al. performed detailed hormonal investigations of healthy and castrated men and concluded that inhibin B is the major regulator of the FSH secretion in men via feedback mechanism (Hayes et al., 2001). In the normal adult male, FSH can stimulate inhibin B production by raising the set point for its production by Sertoli cells without interfering with its diurnal rhythm (Kamischke et al., 2001).

It seems that inhibin regulation in women is even more complex partly due to the existence of inhibin A and inhibin B. The secretion of both is stimulated by FSH in the early follicular phase, when small antral follicles are present (Welt, 2002). FSH and LH stimulate inhibin A from the pre-ovulatory follicle but neither stimulates inhibin B in vivo. This could explain the elevation of the concentrations of inhibin A but not of inhibin B in follicular fluid with increasing follicle maturity (Welt, 2002).

**Association with Y-microdeletion**

Microdeletions of the Y chromosome are responsible for 10–15% of cases of azoospermia and severe oligozoospermia. Inhibin B production in patients with Yq deletions was found to be higher (70%) than in patients without this deletion (Foresta et al., 2001). Frydelund-Larson et al. (2002) reported that the mean serum inhibin B concentration in patients with AZFc (azoospermic factor) microdeletions (39.5 ± 36.0 pg/ml) was significantly lower than that in a group of infertile patients without microdeletions (134.6 ± 88.5 pg/ml). Contradictory results from both these studies need to be confirmed by further trials.

**Changes in blood concentrations of inhibin from birth to adulthood**

Inhibin B is detectable in the umbilical cord blood of male but not female fetuses, and in concentrations similar to those of adult men (Andersson et al., 1998b). During early infancy (3–6 months), serum concentrations of inhibin B in males rise to concentrations higher than those of adult men. Inhibin concentrations in infant girls vary similarly to those in boys (Chellakooty et al., 2003). After 2 years of age, inhibin concentrations decline to the lowest concentrations of childhood (Andersson et al., 1998b).

Concentrations of inhibin B in girls are low until the age of 6 years. They begin to rise at 10–12 years of age and peak between 12 and 18 years (Crofton et al., 2002b). Inhibin A is usually detectable in girls younger than 3 months, but concentrations thereafter become undetectable in most samples until after the age of 10 years. Inhibin A concentrations gradually increase until 14 years of age in girls and then stabilize between 14 and 18 years (Crofton et al., 2002b).

Inhibin B blood concentrations rise with the onset of puberty before there is a detectable increase in testicular volume and the onset of spermatogenesis (Andersson et al., 1997; Crofton et al., 2002a). In adults, however, there is a strong correlation between inhibin B and spermatogenesis. These facts suggest that the regulation of inhibin B production during puberty changes. The positive correlation in early puberty between inhibin B and LH, which corresponds to testosterone, indicates that Leydig cell factors play an important role in the maturation and stimulation of Sertoli cells. Nachtigall et al. (1996) obtained similar results in hypogonadotrophic men treated with GnRH, which is a model of induced puberty.

The relationship between FSH and inhibin B during male puberty and recovery of the hypothalamic–pituitary–testicular axis is parallel to the correlation between FSH and inhibin B during the menstrual cycle.

**Inhibin relation with menstrual cycle**

Blood concentrations of inhibin B appear to vary with the phase of menstrual cycle. Inhibin concentrations increase during the early follicular phase and begin to fall 1 day after the FSH increase until the end of the follicular phase. Inhibin B concentrations rise for a brief period 2 days after the LH peak and decrease to low concentrations during the luteal phase. On the other hand, the concentration of inhibin A is low during the follicular phase, rises during ovulation and reaches its highest concentration in the middle of the luteal phase. The changes in the concentrations of inhibin A and inhibin B during the menstrual cycle suggest that these forms possess different physiological roles (Groome et al., 1996). The peak in inhibin A in the luteal phase and its fall with luteolysis are consistent with its being a secretory product of the corpus luteum.
luteum, as would be expected from the expression of the £-subunit in the corpus luteum (Hayes et al., 2001) (see Figure 2). Penarrubia et al. (2004) reported recently that basal serum concentrations of inhibin B during the early follicular phase seemed to be one of the biomarkers of ovarian reserve. They have noted no difference in inhibin B serum concentrations on cycle day 3 during three consecutive cycles. FSH and inhibin varied less significantly than oestradiol on cycle day 3.

Serum concentrations of inhibin A reach peak during the luteal phase, but its concentration is low during the follicular phase and in post-menopausal women.

**Alterations in inhibin concentrations with age**

In men the concentrations of inhibin B and testosterone decrease as a result of an age-related decrease of testicular function. The concentrations of inhibin B show a weak inverse correlation with the age and the ratio between the blood concentrations of inhibin B and FSH is significantly decreased, because of the moderate decrease of inhibin B and the 4-fold increase of FSH (Mahmoud et al., 2000). Inhibin B may play an important role in the endocrinology of perimenopausal women. Its concentration decreases rapidly in women with normal menstrual cycles between the ages of 35 and 47 years. The concentrations are related to FSH concentrations and might precede changes in oestradiol (Battistini et al., 2002). FSH and LH concentrations are high in women 50 years of age and older (Baccarelli et al., 2001). Inhibin A and inhibin B are undetectable in the ovaries and peripheral blood of post-menopausal women (Ala-Fossi et al., 1998). Concentrations of activin A in men and in women increase with age, especially during the last decades of life when its correlation with FSH does not exist.

**Inhibin B as a marker of exocrine function of the gonads**

Inhibin B is a potentially new marker for testicular exocrine function in reproductive pathology. It could be used with FSH blood concentrations and sperm concentration in the ejaculate as a marker of spermatogenesis. Inhibin B is considered a more direct marker of the Sertoli cell function and spermatogenesis than FSH because the gonadotrophin is an object of complex regulation by the hypothalamic GnRH, steroidal hormones, inhibins, activins and follistatin (Jensen et al., 1997; Hayes et al., 2001). There is a close positive correlation between the number of spermatozoa and blood concentrations of inhibin B, which is similar to the correlation between inhibin B and testicular volume (von Eckardstein et al., 1999). During childhood, inhibin B directly provides information about the existence and function of the testicular tissue and therefore could be used in the diagnostic process of patients with intersexuality and cryptorchidism (Andersson et al., 1997; Andersson, 2000; Lee et al., 2001).

**Inhibins in male infertility**

Sertoli cells secrete inhibin B in response to FSH secretion, and it is the major feedback regulator of FSH secretion. FSH concentrations do not predict the outcome of testicular sperm extraction (TESE) or testicular biopsy. The combination of these two parameters (inhibin B and FSH concentrations) is currently the best predictor for the presence of sperm in TESE.

Bohring and colleagues found that TESE could also be successful when both hormones were outside threshold concentrations (<79 pg/ml for inhibin B and >10 mIU/ml for FSH). Thus, they concluded that the prediction was not absolutely reliable (Bohring et al., 2002). Inhibin B concentrations can be used to monitor the response to gonadotrophin treatment in patients with azoospermia.

Varicocele is considered one of the most important causes of male infertility, and treatment increases the fertility rate. Pierik et al. reported that measurement of basal inhibin B concentrations could provide additional prognostic information on the efficacy of varicocelectomy. Furthermore, it could be used together with other endocrinological predictors including FSH concentrations, androgen concentrations and testosterone response to HCG to predict the success of the surgical intervention more precisely than semen analyses. Inhibin B might help in the assessment of spermatogenesis (Fujisawa et al., 2001; Pierik et al., 2001). In male infertility practice, it is regularly observed that low blood concentrations of inhibin B correspond to elevated serum concentration of FSH.

The importance of inhibin B as a marker of the germ epithelium function, including spermatogenesis, in patients with non-obstructive azoospermia remains unclear. Some authors consider inhibin B blood concentrations to be a sensitive marker for the assessment of sperm production; however, others suggest that it cannot predict the success of spermatozoa extraction from the testis (von Eckardstein et al., 1999; Ballesca et al., 2000; Brugo-Olmedo et al., 2001).

The concentration of inhibin B in the ejaculate is high, even though it may vary significantly in men with normal spermatogenesis (Anderson et al., 1998a). In one study, the basal and reserve activity of Sertoli cells, as judged by inhibin B secretion, was higher in normozoospermic than in dyspermic men, and patients with an adequate response to FSH stimulation had a better therapeutic outcome (Adamopoulos et al., 2003). The fact that semen does not contain inhibin B after vasectomy further confirms the theory that the testis is a predominant source (Anderson et al., 1998a). Interesting and still unexplained is the fact that £-subunits, detectable in all human body fluids, cannot be found in the semen (Anderson and Sharpe, 2000). Activin A and follistatin are found in the male blood circulation. Concentrations of activin A are low in patients with obstructive azoospermia and high in those with other sperm disturbances. High concentrations of follistatin have been measured in semen, but it is still unclear whether they originate from the testes because the same concentrations could be found in healthy men after vasectomy (Anderson et al., 1998b). The concentrations are positively related to age but not with the duration of abstinence, possibly because of the age-related increase of the prostate size. Activin A is also present in the semen (Anderson et al., 1998b) but is undetectable after vasectomy. These data suggest that inhibin, activin and follistatin are important regulators of the seminiferous epithelium function in adults.

Inhibin is a promising tool for monitoring the function of the germinal epithelium and may serve as marker for monitoring...
HCG treatment in patients with infertility in the future. Further research is essential before inhibins can be used in clinical practice.

Inhibins in pregnancy

Inhibin A is mostly secreted from the dominant follicle and the corpus luteum whereas inhibin B is mainly derived from the small antral follicles of the ovary. Inhibin is among immunomodulatory factors that prevent a graft-versus-host reaction. It could be used as a marker of human embryo implantation that may identify defects causing subfertility (Hoozeman et al., 2004). In spontaneous pregnancies, serum inhibin A concentrations begin to rise markedly beginning in the 5th week and peak at 8 weeks of gestation (Illingworth et al., 1996b). Pro-oC containing inhibins mirror that of inhibin A. The placenta and granulosa cells of the ovary are the two main sources of inhibin during pregnancy (Fowler et al., 1998). Inhibin A is the predominant type of inhibin in the first trimester. In the third trimester, however, both A and B types are elevated (Muttukrishna et al., 1995; Petraglia, 1997).

Recently, Muttukrishna et al. (2004) measured concentrations of inhibin, activin and follistatin in the placenta, maternal serum and fetal fluids. They reported that maternal concentrations of serum inhibin A and follistatin were significantly higher than fetal serum concentrations, whereas inhibin B and pro-oC concentrations were higher in the fetal serum in the first trimester. Inhibin B and testosterone were higher in the fetal serum in second trimester, suggesting that these hormones may play a role in the development of the male fetal gonads. A recent case–control study by Wallace et al. revealed that inhibin A, pro-oC and HCG concentrations are significantly lower in failing pregnancies than in normal pregnancies, but the concentrations of activin were not significantly different between these two groups (Wallace et al., 2004).

High-risk pregnancy

Several studies evaluated the role of inhibins in trophoblastic disease. One reported that inhibin concentrations are higher in patients with trophoblastic disease than in healthy controls (Yohkaichya et al., 1989). Another showed that the pro-oC concentration decreased considerably after evacuation of a molar pregnancy (Kato, 2002). Inhibin concentrations fall after the termination of a molar pregnancy, indicating that it can be used as a marker for molar pregnancy. However, because inhibin concentrations begin to rise once ovulation resumes, which does not occur with HCG, measurement of inhibin concentrations may be limited in these circumstances. Inhibin concentrations also fall in women with a non-viable fetus or missed abortions, suggesting that it might be used to help predict whether a pregnancy is viable during early gestation (Lockwood et al., 1997).

Inhibin concentrations are elevated at 15–18 weeks of gestation in patients who subsequently develop pre-eclampsia. Inhibin concentrations are also higher at 21 weeks of gestation in patients with pre-eclampsia who developed the disease at 34–37 weeks than in a control population (Muttukrishna et al., 2000; Muttukrishna, 2004).

Activin A concentrations are also elevated in hypertensive women during pregnancy, and some investigators suggest that they are predictive of subsequent development of pre-eclampsia (Petraglia et al., 1995; Muttukrishna et al., 2000).

The above studies indicate that inhibin can serve as a sensitive marker for placentation function. The exact reason for the higher and lower concentrations in different disorders is not known. However, inhibin may serve as an important diagnostic tool in the future.

While the placenta is thought to be the main source of inhibin during pregnancy, Wallace et al. suggested that the fetal membranes may contribute significantly to the amniotic fluid inhibin A content (Wallace et al., 1997a). In the second trimester of pregnancy, inhibin A increases in the maternal serum in women carrying fetuses with Down’s syndrome. Compared with healthy controls, concentrations of inhibin A, pro-oC inhibin and activin A were found to be significantly lower in the amniotic fluid in Down’s syndrome pregnancies in the second trimester (Wallace et al., 1999). Inhibin A concentrations can serve as a marker for Down’s syndrome between 14 and 19 weeks of gestation.

Lambert-Messerlian et al. concluded that serum inhibin A concentrations were likely to enhance the detection of fetal Turner syndrome with hydrops, but would not contribute substantially to the detection of fetal trisomy 18 (Lambert-Messerlian et al., 1998).

Role of inhibin in polycystic ovarian syndrome

Women with polycystic ovary syndrome (PCOS) are at high risk for over-responding to gonadotrophin stimulation. Inhibin B concentrations are significantly elevated in patients with PCOS (Anderson et al., 1998c; Lockwood et al., 1998). The role played by inhibin in the pathogenesis of PCOS is still unclear. Tanabe et al. (1990) suggested that the increased number of antral follicles in polycystic ovaries increases the potential for inhibin secretion. High oestrogen and the inhibin concentrations may provoke the disparity between basal concentrations of LH and FSH in patients with PCOS. Lockwood et al. reported that inhibin B concentrations in patients with multiple follicular growth was higher than that in those with single follicle development (Lockwood, 2000). Elting et al. reported that FSH-induced inhibin B increments can predict the size of the follicle in regularly ovulating women and in women with PCOS (Elting et al., 2001).

According to Fujiwara et al. (2001), the insufficient production of inhibin A and possibly βB-subunits (but not follistatin) could be associated with the arrest of follicular growth in patients with PCOS. A study by Shelling et al. (2000) showed that the percentage of mutations in the inhibin α and βB subunits genes was higher in women with premature hypo-ovarism versus healthy women.

Role of inhibins in infertility and IVF

Inhibin plays an important role when assessing ovarian reserve. It can be used as a qualitative marker in controlled ovary stimulation and for an early diagnosis of
hyperstimulation syndrome (Dzik et al., 2000). FSH and inhibin B are two independent markers that can predict the number of restored oocytes (Tinkanen et al., 1999). Since serum inhibin B before oocyte retrieval in ovarian hyperstimulation is a strong predictor of the number of oocytes retrieved, it appears to be a useful marker for ovarian response (Fried et al., 2003). Seifer et al. (1997) reported that low concentrations of inhibin during day 3 are associated with poor response to ovulation induction and decreased success during IVF treatment cycles. In additional studies, Seifer et al. (1999) found that women with declining ovarian reserve show evidence of a decrease in day 3 inhibin B concentrations before a rise in day 3 FSH concentrations. According to Lockwood et al. (1997), an unexpected over- and under-response to gonadotrophin stimulation can be predicted by assessing the mid-follicular phase concentrations of inhibin B. Penarrubia et al. (2000) found that basal inhibin B concentrations on day 3 were significantly lower (36.2 ± 8 pg/ml) in the women whose cycles were cancelled than in the control group (49.6 ± 6.9 pg/ml).

Many studies have demonstrated a negative correlation between FSH and inhibin B. Measurement of inhibin B can be useful for assessing ovarian reserve and also predicting the response to ovulation induction agents. Inhibin and pro-oC measurement can be a useful non-invasive tool for the management and counselling of patients who are seeking infertility treatment.

Inhibin A concentrations in the circulation are elevated in normal and IVF pregnancies (Muttukrishna et al., 1995). Illingworth et al. (1996) reported that inhibin concentrations peak at 8 weeks of gestation and then begin to decline. Inhibin concentrations decrease with spontaneous abortion. Thus, measurement of inhibin A during early pregnancy may predict the pregnancy rates in IVF (Muttukrishna et al., 1995).

Inhibins as serum markers for cancers

Inhibins and their free fractions may play a role in various reproductive cancers. The enzyme-linked immunosorbent assays (ELISA) is a simple and specific assay that is more practical for clinical use (Robertson et al., 1999). Researchers have used ELISA to measure inhibin and its free fractions, which led to the discovery that concentrations of inhibin are high in many ovarian and prostate cancers (Robertson et al., 2001).

Ovarian cancers

Robertson et al. (2002a,b) showed that measurement of inhibin containing αC region isoforms provides the best sensitivity and specificity for the diagnosis of ovarian cancers. Some ovarian neoplasms such as germ cell tumours (GCT) give similar profiles with inhibin B and total inhibin assay (Petraglia et al., 1998; Robertson et al., 1999). Several studies reported that GCT are associated with high concentrations of inhibin. These inhibin concentrations normalize after surgical resection of tumour, indicating that these tumours secrete high concentrations of inhibin, which can serve as a serum marker. Some of these studies also found a correlation between the rise of inhibin concentrations and tumour recurrence in the post-operative period (Jobling et al., 1994; Cooke et al., 1995; Boggess et al., 1997).

Inhibin and CA125 (marker currently used for epithelial tumours) have both been found to be highly specific markers for the GCT. Robertson et al. (2002a,b) combined both the measurements in post-menopausal women found that combination of both the tests increases the detection rates from 82 to 95%.

The inhibin α subunit has found to be elevated in sex cord stromal tumours (SCST) of the ovaries. Monoclonal and polyclonal antibody directed against the NH2 region of the αC fragment of α subunit can serve as an immunocytochemical marker for SCST (McClimagge, 2002; Zheng et al., 2003). Epithelial tumours constitute the majority of ovarian cancers. Mucinous cancers are associated with the free α subunit of ovarian cancers. Healy et al. observed that serum inhibin concentrations were elevated in eight of nine women with mucinous cystadenocarcinomas (Healy et al., 1993).

Inhibin is normally produced in premenopausal ovaries, which keeps plasma concentrations high. Inhibin may be useful in post-menopausal women with GCTs and mucinous tumours. Serum activin concentrations are elevated in almost three quarters of women with epithelial ovarian cancers but decrease to near normal concentrations after surgical excision of the tumour (Lambert-Messerlian et al., 1999).

Other cancers

Inhibin subunits are expressed on normal prostate and its concentrations are lower than normal in malignant tissue. The latter observation led to the assumption that inhibin subunit expression was suppressed in malignant tissue (Risbridger et al., 2001).

The inhibin α subunit has been localized on Sertoli cell and Leydig cell tumours of the testis. However, there is no detectable immunoactivity in germ cell tumours. The usefulness of inhibin as a diagnostic marker for either Leydig cell or Sertoli cell tumours has not been assessed (Iczkowski et al., 1998).

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