Review

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 pro-αC 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 N-αC and pro-β-β) (Illingworth *et al*., 1996a). There are two forms of β subunits, β_A and β_B. The complex $\alpha\beta_A$ is called inhibin A, and the complex $\alpha\beta_B$ 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 α/β structure. Only dimeric forms of inhibin are biologically active. The normal range of inhibin B estimated by enzyme immunometric assay is variable.

Activins

Activin A is a homodimer of β_A-subunits ($\beta_A\beta_A$), whereas activin B is a homodimer of β_B subunits ($\beta_B\beta_B$) and activin AB is a heterodimer of β-subunits ($\beta_A\beta_B$) (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.

α_2-Macroglobulin

α_2-Macroglobulin (α_2-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. α_2-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 β_B-subunits, Andersson *et al*. established that the germ cells, but not the Sertoli cells, could be immunostained for β_B. The Sertoli cells contain only the α-subunits where as the β_B-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; Anderson, 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 β_B-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; Anderson *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 (Kinniburgh and Anderson, 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 β_B-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*., 1992; 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). Adrenocorticotrophic 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 spermatic 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; Kogawa *et al*., 1991). It has also been found in the spermatic fluid, where its concentration after vasectomy remains unchanged. These data suggest that prostatic epithelium and seminal vesicles can release follistatin in the spermatic 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 (Carlsen *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_B-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 cell 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

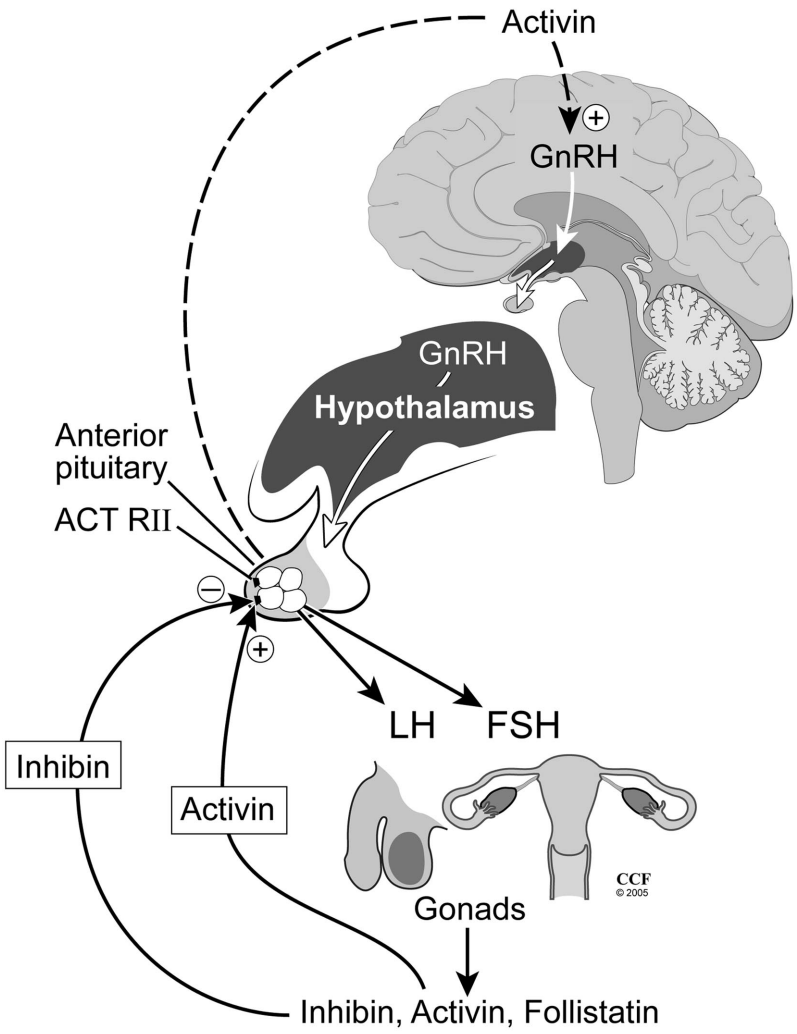

Figure 1. Schematic mechanism of regulation of inhibin and activins on gonadotrophin secretion. Activin and follistatin are synthesized by the gonads, pituitary, and hypothalamus. Activin binds to activin receptor type II (ACT RII) in the gonadotroph cells of pituitary and increase the secretion of FSH and LH. Inhibin is released by the gonad and acts in a classical endocrine manner and negatively regulates the activin stimulation of FSH. Inhibin binding to the same receptor (ACT RII) inhibits the secretion of FSH and LH. The preliminary evidence suggests that activins appears to stimulate the gonadotrophin releasing hormone (GnRH) release in the hypothalamus.

concentrations from the healthy male controls (Nachtigall *et al*., 1996; Seminara *et al*., 1996).

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

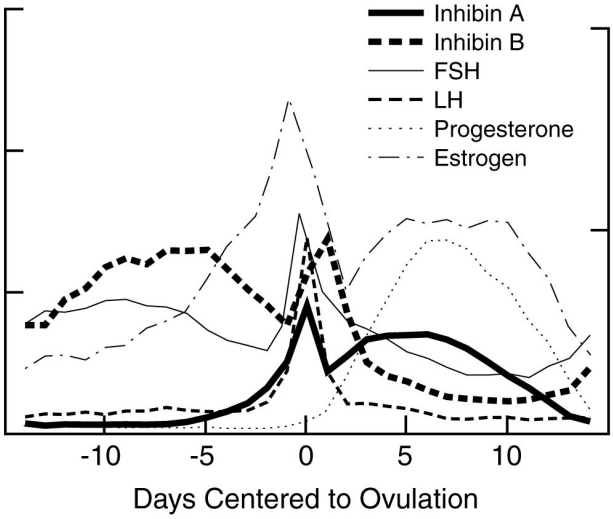

Figure 2. The changes in inhibins and other hormones during the menstrual cycle are shown. Inhibin B concentration increases across the luteal-follicular transition reaching peak in mid-follicular phase and a second peak immediately after LH surge. Inhibin A begins to rise in the late follicular phase reaching peak in mid-cycle and subsequently in the mid-follicular phase. Units are arbitrary.

luteum, as would be expected from the expression of the β_A-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 reaches 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 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 α-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 (Hoozemans *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-αC 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-αC 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-αC 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 (Yohkaichiya *et al*., 1989). Another showed that the pro-αC 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 placental 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-αC 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 β_A-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 β_B 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-αC 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 NH_2 region of the αC fragment of α subunit can serve as an immunocytochemical marker for SCST (McCluggage, 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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