INVITED REVIEW

Inhibin B in male reproduction: pathophysiology and clinical relevance

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Abstract

The recent availability of specific inhibin assays has demonstrated that inhibin B is the relevant circulating inhibin form in the human male. Inhibin B is a dimer of an α and a β_B subunit. It is produced exclusively by the testis, predominantly by the Sertoli cells in the prepubertal testis, while the site of production in the adult is still controversial. Inhibin B controls FSH secretion via a negative feedback mechanism. In the adult, inhibin B production depends both on FSH and on spermatogenic status, but it is not known in which way germ cells contribute to inhibin B production. The regulation of inhibin B production changes during life. There is an inhibin B peak in serum shortly after birth only partly correlated with an increase in serum FSH, probably reflecting the proliferating activity of the Sertoli cells during this phase of life. Afterwards, inhibin B levels decrease and remain low until puberty, when they rise again, first as a consequence of FSH stimulation and then as a result of the combined regulation by FSH and the ongoing spermatogenesis. In the adult, serum inhibin B shows a clear diurnal variation closely related to that of testosterone. The administration of FSH increases the secretion of inhibin B in normal men, but is much more pronounced in males with secondary hypogonadism. The treatment of infertile men with FSH, however, does not result in an unequivocal inhibin B increase. There is a clear inverse relationship between serum inhibin B and FSH in the adult. Serum inhibin B levels are strongly positively correlated with testicular volume and sperm counts. In infertile patients, inhibin B decreases and FSH increases. In general, there is very good correlation with the degree of spermatogenetic damage, with the arrest at the earlier stages having the lowest inhibin B levels. However, for unknown reasons, there are cases of Sertoli-cell-only syndrome with normal inhibin B levels. Inhibin B and FSH together are a more sensitive and specific marker for spermatogenesis than either one alone. However, the inhibin B concentrations are not a reliable predictor of the presence of sperm in biopsy samples for testicular sperm extraction. Suppression of spermatogenesis with testosterone and gestagens leads to a partial reduction of inhibin B in serum but it is never completely suppressed. In contrast, testicular irradiation in monkeys or humans leads to a rapid and dramatic decrease of inhibin B, which becomes undetectable when germ cells are completely absent. In summary, although inhibin B is a valuable index of spermatogenesis, the measurement of serum inhibin B levels is still of limited clinical relevance for individual patients.

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Introduction

The existence of a testicular, non-steroidal, endocrine factor controlling pituitary function was postulated in 1932, around the time of gonadotrophin discovery and isolation, when McCullagh (1) reported that aqueous testicular extracts prevented the formation of 'castration cells' in the rat pituitary. Moreover, McCullagh was able to show that the selective destruction of the seminiferous epithelium was sufficient to cause pituitary hyperfunction without inducing atrophy of the secondary sex glands, i.e. hypoandrogenism (1). Thus,

in this early period, inhibin (latin *inhibere*, to suppress) was defined in its essence: a non-steroidal testicular substance, produced by the seminiferous epithelium, specifically inhibiting follicle-stimulating hormone (FSH) secretion. Decades of research have led to the discovery and characterisation of a large family of proteins, the inhibin family (2, 3), and to the partial definition of the function of its members. However, it was only the development of specific immunoassays for biologically active, dimeric forms of inhibin that provided new insights into our understanding of the physiological relevance of inhibin in male reproductive

function. Today, it is clear that inhibin B is the only inhibin form present in male circulation. Serum inhibin B levels reflect the functional state of the seminiferous epithelium and are involved in the feedback of the pituitary–gonadal axis, as shown by many clinical studies investigating inhibin B in a variety of physiological and pathological states. Although these studies have provided insight into inhibin biology, many aspects of inhibin B synthesis, secretion, function and mechanism of action remain elusive and, sometimes, contradictory. Perhaps not unexpectedly, inhibin B serum levels correlate quite well with FSH concentrations in the vast majority of cases and the measurement of serum inhibin B, initially regarded as the long-awaited tool which could render obsolete more invasive diagnostic interventions, has not yet found unequivocal application in clinical routine. Nevertheless, the possibility of measuring serum inhibin B has enriched our knowledge of male gonadal function. The primary aim of this review is to discuss the recent clinical data that have added to our understanding of inhibin pathophysiology and the usefulness of inhibin B as a diagnostic tool in the assessment of testicular function.

Inhibin B structure, synthesis, secretion and action

Inhibin is a glycoprotein hormone of gonadal origin which was first identified by its ability to negatively regulate FSH (4). Inhibin consists of two covalently linked subunits, a common α subunit and a β subunit, the latter of which exists in two forms, A and B (denoted β_A and β_B respectively). Although many molecular forms are found in circulation, biological activity resides only in the dimeric forms, inhibin A ($\alpha\text{-}\beta_A$) and B ($\alpha\text{-}\beta_B$) (5).

Over the last three decades there have been numerous studies on inhibin synthesis and secretion in a number of *in vivo* and *in vitro* models; however, such studies failed to demonstrate an unequivocal or systematic association between serum inhibin levels and spermatogenesis (6, 7). One reason for this was the lack of specific and sensitive assays to measure the variously sized inhibin forms. Levels of inhibin in blood were measured using heterologous assays, e.g. the Monash radioimmunoassay (6) which could not distinguish between the bioactive, dimeric inhibin form ($\alpha\text{-}\beta_A$ and $\alpha\text{-}\beta_B$) and the inactive forms such as free α subunit (pro- $\alpha\text{N}\text{-}\alpha\text{C}$) and its precursor (pro- αC)-related immunoactive peptides (8, 9). However, the production of highly specific antibodies and the advent of a new two-site, enzyme-linked immunoabsorbent assay (ELISA) for specific measurement of the bioactive inhibin A and B (10) has overcome this problem and has led to the realisation that inhibin B is the major form produced in the foetal and adult human male, where inhibin A is undetectable (8, 11–13). This is in

agreement with animal studies including the rhesus monkey (14) and the rat (15).

The Sertoli cell is considered the predominant source of inhibin B (16, 17). This notion is currently challenged and confounded by a mounting number of sometimes contradictory studies, based on immunolocalisation of inhibin subunits and on the *in vitro* and/or *in vivo* production of inhibin B in various experimental settings. These studies indicate that germ cells and possibly even Leydig cells would produce inhibin as well (18, 19). The human male inhibin subunits seem to be differentially localised and secreted depending on age and cell type. For example, in the foetal testis, α - and β_B subunits, but not β_A were immunolocalised in the Sertoli and Leydig cells (20). In cultured testicular cells from prepubertal boys, both highly purified luteinizing hormone (LH) and recombinant FSH stimulated inhibin B secretion (21). In the adult human testis, β_A and β_B subunits were found in Sertoli and Leydig cells but not in germ cells (22). In a recent intriguing study, positive staining for the inhibin α subunit was evident in Sertoli cells, while β_B subunit was evident in germ cells from pachytene spermatocytes to early spermatids but not in Sertoli cells (19), suggesting that the two subunits that constitute inhibin B could be contributed by different cell types in adult men. Electron microscopic evidence reports the transfer of inhibin α subunit from Sertoli cells to spermatocytes in the human testis (23), suggesting that inhibin subunit dimerisation could occur in the spermatocytes. Extracellular dimerisation may also take place (24). In any case, it should be considered that the different antibodies used in these studies are recognising different epitopes which could be shared by intermediate products of the inhibin family and/or they could identify β subunits destined to become part of activins. The development of an activin B immunoassay, not available at the moment, should help in resolving this question.

If the α and the β_B subunits localised in Leydig cells are assembled into mature inhibin B, Leydig cells should contribute to the levels of inhibin B in circulation. However, administration of recombinant LH to hypogonadal men or human chorionic gonadotrophin to normal men is unable to raise serum inhibin B levels, demonstrating that, in man, Leydig cells do not contribute to the pool of circulating inhibin B (25, 26). In summary, although the contribution of germ cells in the regulation of inhibin B secretion is no longer subject to question (see below), Sertoli cells should still be considered to be the main source of inhibin B in the human male, until its assembly and secretion by other cell types is unequivocally proved.

The route of secretion of inhibin B in the bloodstream is still poorly understood. Inhibin B is secreted in seminal plasma and it has been shown that Sertoli cells secrete inhibin in a highly vectorial manner. In cultured primate Sertoli cells >90% of inhibin is

secreted into the adluminal compartment in the presence of FSH (17). Studies in the rat have shown that the major route via which inhibin reaches the bloodstream is through secretion into the seminiferous tubular fluid, thus supporting the idea of intratubular production of inhibin (27). The presence of bioactive inhibin in seminal plasma has been long recognised (28). Indeed, one of the first studies supporting the inhibin hypothesis showed that the subcutaneous injection of seminal plasma from normal men into castrated rats significantly inhibited serum FSH, while seminal plasma from azoospermic men did not (29). Seminal plasma of normozoospermic men contains high but extremely variable levels of inhibin B (from undetectable up to >50 000 pg/ml) while inhibin is undetectable in vasectomised patients (13, 30). The reason for this large variability in seminal plasma concentrations in the presence of rather constant serum levels is unknown, but suggests that the transport of inhibin B into the blood might involve some active, yet unknown mechanism.

Serum inhibin B shows a clear diurnal rhythm parallel to that of testosterone. Individual diurnal profiles of serum inhibin B in 13 subjects, in whom frequent serum samples were withdrawn continuously for 24 h, suggested that testosterone and oestradiol might play a role in the diurnal rhythm of inhibin B, which is independent of FSH (31). In fact, administration of a high FSH dose (3000 IU) to healthy volunteers results in a significant increase of serum inhibin B which, however, maintains its typical diurnal rhythm with the lowest concentrations in the evening/night hours (32). Since circadian testosterone fluctuations are independent of LH as well, both inhibin B and testosterone rhythms could be regulated by the same, gonadotrophin-independent, local mechanism.

The main function of dimeric inhibin (both A and B) is the negative control of FSH secretion (33). In the mouse, inhibin might have an intratesticular action, e.g. in the control of Sertoli and Leydig cell neoplastic proliferation (34), but there is no evidence of any clinically relevant, intratesticular inhibin action so far. The mechanism by which inhibin B exerts its biological effect is still the subject of intensive research. Inhibin has been shown to bind to the activin type II receptor (ActRII) and thereby inhibit activin action. However, inhibin does not antagonise activin action in all the tissues in which this hormone acts, suggesting the existence of a specific binding factor required for inhibin action. In addition, evidence has accumulated that, besides being an activin antagonist, inhibin has specific binding sites in tissues where it is thought to act, such as the pituitary (35). Indeed, two putative, specific inhibin receptors have been isolated and molecularly characterised last year: betaglycan and p120 (36, 37). Betaglycan, also known as type III transforming growth factor- β receptor, functions as inhibin co-receptor in the presence of ActRII, resulting in enhanced antagonism of activin action (36). p120,

isolated with a classical biochemical approach from bovine pituitaries based on its capacity to bind inhibin A, is localised to the gonadotropes of the pituitary gland and Leydig and Sertoli cells (37). p120, also called inhibin-binding protein, is a specific inhibin B receptor and, via association with the type IB activin receptor, appears to be necessary for inhibin B-induced antagonistic effect on activin-stimulated signal transduction (38).

Control of inhibin B secretion

The first specific assay for the measurement of serum inhibin B was available in 1996 (39). After the first demonstration that inhibin B is the physiologically relevant inhibin form in man (8), this assay was used to study the changes in serum inhibin B levels in a number of pathophysiological conditions, such as maturation and senescence, infertility, hypogonadism, gonadotrophin administration, hormonal contraception and testicular damage resulting from radiation or chemotherapy. All these studies provided very relevant, although sometimes contradictory, information about inhibin physiology, explaining in part the complex interplay between FSH, inhibin B and spermatogenesis. It should be considered that all the data published so far have been obtained using only one assay, which still has a rather poor interassay precision (Fig. 1) and cannot be properly evaluated in terms of accuracy since internationally recognised reference preparations are not available. Considering the extreme heterogeneity of immunoreactive species of inhibin in the circulation, appropriate standardisation is crucial and the availability of alternative assays would be very valuable.

Childhood and pubertal maturation

Serum inhibin B levels as well as the relationship between FSH and inhibin B vary throughout life in the human male, with a major switch in inhibin regulation

Figure 1 Measurement of serum inhibin B by a commercially available ELISA. Results from the measurement of control serum with normal inhibin B values in 111 consecutive assays are shown. The interassay coefficient of variation was 18.9%.

occurring around puberty. While Sertoli cell proliferation and FSH govern inhibin activity in childhood, germ cells are a major determinant of inhibin B production in adulthood.

Inhibin B levels are low but detectable in cord blood and increase into the adult range as early as in the first week after birth in male newborns (40), continue to rise up to levels higher than those observed in adults by 3–6 months of age (30, 41, 42) and then decline progressively to reach their nadir towards 3–6 years (30, 42). This early postnatal increase is presumably due to the activation of the hypothalamic–pituitary testicular axis (41) and mirrors the proliferating activity of Sertoli cells. Intriguingly, the early postnatal rise of inhibin B is better correlated with LH and testosterone than with FSH (42, 43), raising the possibility that Sertoli cell proliferation in neonatal life depends more on LH/testosterone than on FSH. Inhibin B levels remain relatively high for several months while FSH concentrations are low, suggesting that the negative feedback control of FSH secretion might already be established between the first and second years of age and that inhibin B production, probably initially activated by FSH and/or by LH, continues without gonadotrophin stimulation under the influence of yet unknown factors (41). It has been suggested that inhibin B might be a marker of the presence of the testis in case of sexual ambiguity with cryptorchidism but no studies have been carried out yet to verify this hypothesis. Inhibin B can be elevated by recombinant FSH administration in prepubertal hypogonadal boys (44).

After the initial postnatal rise, serum inhibin B levels are low until puberty but still higher than in adults with impaired spermatogenesis. This suggests that the regulation of inhibin B secretion in childhood is different from that in the adult. A critical change in the regulation of inhibin B secretion occurs at puberty (45) or just prior to mid puberty (44). Prepubertal boys lacking spermatogenesis and the relevant germ cell types secrete inhibin B in readily measureable amounts (41, 46), whereas adult men with impaired spermatogenesis (Sertoli-cell-only; SCO) have low to undetectable levels of serum inhibin B in the presence of elevated FSH concentrations (11). Obviously, prior to puberty, inhibin B levels are independent of the presence of actively proliferating germ cells, while after puberty serum inhibin B levels are closely related to the spermatogenic status (41). During puberty, the main control of inhibin B secretion switches from FSH to spermatogenesis. Basal inhibin B increases under FSH stimulation in the first pubertal stages when the last wave of Sertoli cell proliferation occurs and a positive correlation between inhibin B and FSH is observed. At Tanner stages G3 and G4, however, FSH and inhibin B levels correlate negatively, suggesting that the negative feedback regulation loop is fully established at this stage of development (46–48).

Altogether, the existing data suggest that the stimulatory activity of FSH on inhibin B is the predominant form of control as long as Sertoli cells proliferate (49–52), although it is not known whether LH can also influence inhibin B production in childhood and puberty. At puberty, when Sertoli cell proliferation ceases and spermatogenesis starts, the basal, adult inhibin B level is set and can be considered to be an index of Sertoli cell density. Once full spermatogenesis is ongoing, changes in inhibin B levels reflect mainly the status of germ cell proliferation and development and depend only secondarily on FSH. Therefore, the main regulation of inhibin B production seems to change at puberty. For instance, puberty could induce a switch in the control of inhibin subunit expression, and it has been postulated that testosterone might be responsible for this maturational switch (53) in early pubertal boys (47).

Adulthood

In the adult, FSH stimulates the production of inhibin B in the testis and inhibin B inhibits the secretion of FSH. Clinical findings show a strong inverse correlation between inhibin B and FSH in healthy men and in men with testicular disorders (8, 11, 54–56). However, unlike the LH–testosterone axis, the production of inhibin B is not only dependent on FSH. After puberty, in the presence of FSH, the prime regulator of inhibin B levels is the spermatogenic status and inhibin B production is directly proportional to the ‘amount’ of spermatogenesis, as shown by the direct correlation between serum inhibin B and sperm count (57). When spermatogenesis is damaged, as in SCO syndrome or after testicular irradiation, inhibin B falls and FSH increases. Under these circumstances, Sertoli cell function might be altered as well, at least because, in the absence of germ cells, Sertoli cells cannot fulfil their primary function. In any case, although FSH stimulates inhibin B secretion in normal men (11), inhibin B production is resistant to the endogenous, elevated FSH levels in infertile men. Therefore, FSH stimulation of inhibin B secretion in the adult is mediated by and necessitates the presence of germ cells. It is possible that, in the adult, FSH stimulates the α subunit and thereby the secretion of inhibin B, provided that enough β_B subunit is available: the availability of β_B subunits for assembly into the dimeric, bioactive form would be the limiting factor under the control of the germ cells. In fact, the transcription of the α and β_B subunit genes is regulated by different mechanisms (58) and FSH stimulates the α subunit mRNA expression but not that of the β_B subunit (59). Moreover, FSH stimulates pro- α -C secretion (11, 26) and pro- α -C is elevated in infertile men while inhibin B is low (6, 10). Expression of the gene encoding inhibin α is upregulated through a cAMP mechanism (58, 60) whereas the inhibin β_B gene does not have a classical

cAMP response element (61) and is not markedly influenced by hypophysectomy or FSH stimulation (62) in the rat. Thus a decrease in the inhibin B feedback signal results in a sustained increase in FSH secretion which, in turn, directly stimulates inhibin α . This hypothesis explains the lack of correlation between FSH and inhibin originally described using the Monash assay (6) which crossreacted with inhibin precursors containing the α subunit.

Which germ cell type is responsible for the regulation of inhibin B? Studies in the rat report that inhibin secretion from Sertoli cells is regulated by interaction with pachytene spermatocytes and early spermatids (63, 64). However, in the primate, there is evidence to suggest that spermatogonia may also modulate inhibin B. Arrest of spermatogonia proliferation in irradiated monkeys was associated with a rapid and marked decrease of inhibin B in the presence of later germ cell types (65) and normal inhibin B levels have been found in a patient presenting with Reifstein's syndrome in whom spermatogonia were the only germ cell type present (55). As a general rule, inhibin B concentrations are progressively lower in groups of men with increasing spermatogenic damage with undetectable inhibin B in azoospermic men with SCO syndrome (8, 55, 66). However, in many individual cases it is not possible to show any clear-cut relationship between spermatogenic cell type, inhibin B and FSH (55, 66). It might be that other, still unrecognised factor(s) take part in the complex interplay between FSH, inhibin B and spermatogenesis, which makes inhibin B a rather unpredictable clinical marker for spermatogenesis in individual patients (see below).

Clinical significance of inhibin B measurement

The demonstration that inhibin B is the relevant inhibin form in man prompted a number of clinical investigations aimed at the analysis of this 'new' endocrine parameter in several pathophysiological settings. To what extent is inhibin B a reliable and clinically useful biomarker of spermatogenesis? Does it have any prognostic value?

Response to FSH stimulation

Is inhibin B a pharmacodynamic parameter of FSH action, i.e. a marker of FSH-dependent Sertoli cell function? If this is the case, it should be possible to identify prospectively a subgroup of infertile patients who might benefit from FSH treatment. It has been shown that FSH (3000 IU) induces a significant increase of serum FSH within 24 h from injection in normal men (11). Such an increase is partially dose dependent, and 1000 IU FSH are sufficient to stimulate inhibin B secretion significantly (32). However, in normal men, 225 IU recombinant FSH, i.e. a dose

capable of increasing inhibin B concentrations in normal women (67), did not result in any appreciable rise in serum inhibin B values (26). No acute FSH stimulation test has been performed yet in men with idiopathic infertility and normal or slightly elevated FSH levels and the chronic treatment of infertile patients yielded conflicting results. In a placebo-controlled study, FSH (150 IU daily for 12 weeks) administration could not significantly increase inhibin B production in infertile men and no improvement in semen parameters or pregnancy rate was observed (68). On the other hand, when 11 patients with oligozoospermia, moderate hypospermatogenesis and normal FSH and inhibin levels were treated with FSH (75 IU every second day for 3 months), inhibin B increased significantly in serum but sperm concentration improved only in six men (69). Therefore, in infertile men as a group, serum inhibin B does not seem to be of much predictive value.

Hypogonadotropic hypogonadism

A few studies have been dedicated to the study of the effects of gonadotrophin-releasing hormone (GnRH) or gonadotrophin treatment on inhibin B secretion in hypogonadotropic hypogonadism. Serum inhibin B levels are in the prepubertal range in men with isolated GnRH deficiency and increase significantly into the normal adult range during pulsatile treatment (56, 70). Similar to that observed during physiological puberty, the negative feedback relationship between inhibin B and FSH is evident during treatment as demonstrated by the establishment of a negative correlation between these two parameters (56). Long-term GnRH treatment does not further increase inhibin B levels, again demonstrating that, beside FSH, local testicular factors are involved in the regulation of inhibin B secretion (70). A recent study demonstrated that the gonadotrophin effect on inhibin B secretion is entirely due to FSH and that Leydig cells do not contribute to the pool of circulating hormone. Recombinant FSH administration (150 IU/day for 1 month) to patients with acquired hypogonadotropic hypogonadism was able to increase serum inhibin B levels progressively, while recombinant LH had no effect (25). Together with the data on FSH stimulation in normal men (11, 32), these results show that FSH and not LH stimulates inhibin B production. However, when spermatogenesis is normal, large supraphysiological doses of FSH are necessary in order to raise the inhibin B values further (11, 26, 32).

Male infertility

To date the only predictors of the state of spermatogenesis are semen analysis, the measurement of FSH in blood and testicular histology. The diagnostic accuracy of FSH is limited by the fact that spermatogenic arrest

at late stages does not lead to changes in FSH secretion and that FSH may be normal even in patients with focal SCO or hypospermatogenesis (71). In fact, serum FSH is not an absolute parameter for the selection of azoospermic men as candidates for testicular sperm extraction (TESE) in assisted reproduction (72, 73). Testis biopsy is an invasive procedure associated with potential complications (74). In addition, a biopsy may not be representative for the whole testis (75). Multiple biopsies which are performed for TESE often show a large variation in the completeness of spermatogenesis. This heterogeneity is even more conspicuous in patients with impaired spermatogenesis, where sections with complete spermatogenesis may be found along with others with focal spermatogenesis (71). Given these limitations, any demonstration that inhibin B can discriminate between complete absence of germ cells in the testis and less severe disturbances of sperm production would be of considerable clinical value.

Indeed, it has been shown that inhibin B serum levels represent a good marker of spermatogenesis which correlates with FSH, testis volume, sperm counts and the presence of SCO tubules (30, 41, 54–57, 69, 76–78). In particular, when used in combination with FSH, inhibin B is a more sensitive marker than FSH alone (55, 77). However, inhibin B, alone or in combination with FSH, cannot predict the presence of sperm in individual testicular tissue samples (55). Moreover, inhibin B cannot predict accurately the type of spermatogenic damage. For instance, in cases of late spermatogenic arrest, inhibin B concentrations may remain normal, exactly like FSH (66). In addition, in a considerable number of cases of non-obstructive azoospermia with focal SCO, both inhibin B and FSH are in the normal range (55, 66). Therefore, despite the fact that the combination of inhibin B and FSH represent a more sensitive predictor of the spermatogenic state than either of them alone, inhibin B has not dramatically improved the diagnostic and prognostic value of FSH for individual patients.

This has important implications for TESE. We have investigated the diagnostic sensitivity of inhibin B in successful retrieval of sperm in 52 TESE patients. Although the combination of serum FSH and inhibin B measurements showed high diagnostic sensitivity (75%) and specificity (73%) in identifying patients in whom sperm could be possibly retrieved, no sperm could be retrieved in 25% of the cases with normal FSH and normal inhibin B. On the other hand, sperm could be extracted in 38% of the cases with inhibin B values <20 pg/ml. Therefore, inhibin B alone or in combination with FSH cannot be used to decide if a patient should undergo TESE (55). At odds with our observation, a recent study has suggested a cut-off of 40 pg/ml as fully predictive of the presence of sperm upon TESE (79). However, the low number of subjects analysed (17 vs 52 in our study) and the high variability of the current inhibin B assay, especially at such low levels,

recommend caution. Many reports have suggested that recovery of testicular spermatozoa may be possible in >50% of cases of non-obstructive azoospermia regardless of clinical parameters such as testicular size or serum FSH (73, 75, 79, 80) and that the success rate is increased by collecting more tissue (81). Even if inhibin B is useful, the clinical decision as to whether TESE should be performed or not cannot be based on inhibin B, either alone, or in combination with FSH and other parameters.

Radiation and antineoplastic therapy

Elimination of the seminiferous epithelium through radiation is followed by an increase in serum FSH levels. An experimental study in cynomolgus monkeys has recently shown that radiation is immediately followed by a sharp drop in serum inhibin B values which occurs well before the decrease in testis volume and semen count and the increase in serum FSH (65). Therefore, in this experimental model, inhibin B is an early marker of testicular injury, most probably an index of premeiotic germ cell proliferation. This experimental observation in the monkey, however, has not yet been followed by similar investigations in the human. It is well known that even before orchiectomy patients with testicular cancer have impaired spermatogenesis (82) which deteriorates even further after surgery (83). In this clinical setting, inhibin B levels also correlate quite well with the spermatogenic status and undetectable inhibin B levels are associated with an absence of spermatogenic activity (84). Similarly, treatment with radioiodine for thyroid carcinoma (85) or chemotherapy (12, 86) are followed by a decrease in spermatogenesis which is reflected by decreased inhibin B and increased FSH levels. Whether inhibin B is a more sensitive and precocious parameter of the damage of the seminiferous epithelium than FSH has not been investigated. However, on the basis of the results obtained in monkeys after irradiation (65) or hemiorchiectomy (87) it would seem that inhibin B could be useful as an early marker of testicular toxicants.

Ageing

Few data are available on spermatogenesis in older men, particularly in men over 60. Controlled studies suggest that semen parameters do not necessarily deteriorate as an effect of ageing in healthy men (88). Recent studies have shown only a moderate decrease in serum inhibin B in old men (30, 89) but, in the absence of semen analysis, this decrease cannot be attributed to the ageing process *per se*. Rather, similar to all other clinical conditions examined until now, a decrease in serum inhibin B could reflect a decrease in spermatogenesis for whatever reason (90).

Figure 2 Inhibin B levels in the serum of male volunteers undergoing trials of hormonal male contraception. Upper panel: volunteers ($n = 6$) were treated with the GnRH antagonist Cetorelix (dose 10 mg/day for 5 days, followed by 2 mg/day up to week 12) for 12 weeks and 19-nortestosterone (400 mg in week 1, followed by 200 mg every 3 weeks) for 28 weeks (95). Lower panel: volunteers received either testosterone undecanoate (1000 mg every 6 weeks) and placebo (■) or testosterone undecanoate plus norethisterone enanthate (200 mg every 6 weeks) (□) (96). The arrows indicate the duration of the treatment. Results are expressed as means $\pm$ s.d. No significant change in serum inhibin B levels could be detected over the treatment and observation periods.

Hormonal male contraception

Current regimens of hormonal male contraception are based on administration of testosterone esters possibly combined with a gestagen. This treatment leads to rapid suppression of serum gonadotrophins and variable suppression of spermatogenesis with azoospermia being induced in about 60–90% of the Caucasian population, mainly depending on the type of gestagen. Some studies have investigated the concentrations of inhibin B in serum (11, 91–94) and in seminal plasma

(94) in men treated with hormonal contraceptives. However, the contradictory results of such studies are puzzling. The administration of testosterone enanthate (TE) for 65 weeks was followed by a rapid and progressive decline in serum inhibin B concentrations which continued until the end of the treatment (92). A similar, significant decline was already observed in another study of 19–24 weeks of TE administration (91). The treatment with TE plus levonorgestrel for 6 months was followed by a significant decline of serum inhibin B in one study (11), while, in our experience, transdermal testosterone plus levonorgestrel for 22 weeks was not followed by a significant decline in inhibin B (93). Finally, treatment with testosterone pellets plus different doses of desogestrel for 8 weeks did not result in any significant change of inhibin B in serum, while the hormone was dose-dependently suppressed down to undetectable levels in seminal plasma (94). Interestingly, in the latter study, serum pro- α -C levels were significantly decreased, again supporting the gonadotrophin dependency of this parameter. In clinical trials of male contraception based on testosterone undecanoate and levonorgestrel or norethitesterone enanthate (95, 96), we could not find any significant decrease in serum inhibin B concentrations, despite a profound suppression of gonadotrophins and spermatogenesis (Fig. 2). These contradictory results are difficult to reconcile, but differences in the duration of treatment, in the type of gestagens and in their mode of administration might play some role. Treatment with TE alone has been reported to affect mainly type B spermatogonia which stop proliferating as a result of gonadotrophin suppression (91). If spermatogonial proliferation is the main determinant of inhibin β_B production, a decline in inhibin B has to be expected. The situation might be different if a gestagen is added, which could have some direct effect on inhibin β_B transcription and/or might lead to a spermatogenic arrest at later stages, not directly influencing inhibin B production. The effects of long-term treatment (beyond 1 year) are not known. In any case, the original hope of distinguishing between responders and non-responders to hormonal contraceptive regimens based on inhibin B determination remains unfulfilled and the dissociation between serum inhibin B concentrations and sperm count in many studies remains an enigma.

Conclusion

The development of a specific and sensitive assay for the measurement of inhibin B has led to significant breakthroughs in our understanding of inhibin B biology and control of FSH secretion (97). Yet its clinical usefulness is rather disappointing and the measurement of this hormone in clinical samples does not significantly reinforce the diagnostic arsenal of the clinical andrologist. Although FSH and inhibin B together are more sensitive than either one alone in

predicting the histological status of the testis and/or the presence of sperm in bioptic tissue, this parameter gives no absolute certainty. Concerning diagnostic and therapeutic recommendations for the individual patient, inhibin B measurement does not really add much to FSH values, semen analysis and clinical evaluation of the testis. On the contrary, inhibin B values are sometimes at odds with the other parameters and can generate interpretative uncertainty. Moreover, it is still unclear whether inhibin B can be used as a functional parameter of Sertoli cells. Finally, inhibin B is not predictive of FSH responsiveness in idiopathic infertility and cannot discriminate between responders and non-responders in trials for hormonal male contraception. Thus, inhibin B is not an indispensable diagnostic tool in clinical practice.

On the other hand, the measurement of inhibin B is very useful in experimental studies and can still add important information to our knowledge of testicular function and regulation of the pituitary–gonadal axis in several pathophysiological conditions. For instance, inhibin B seems to be a very fast marker of testicular damage and could become important for rapid identification of spermatogenic disorders in populations exposed to testicular toxicants. Moreover, many enigmatic aspects of inhibin B physiology remain to be clarified and deserve further investigation. Finally, methodological aspects should not be disregarded. We should keep in mind that the totality of the data on which our current concept is based has been generated using only one and the same assay kit. Several decades of experience with other protein hormone assays suggest that standardisation and availability and marketing of new, competing assay kits could hold some surprises in store.

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