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DEBATE

Increased follicle stimulating hormone in infertile men

Is increased plasma FSH always due to damaged germinal epithelium?

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In infertile men, a raised concentration of follicle-stimulating hormone (FSH) is considered a reliable indicator of germinal epithelial damage and is usually associated with azoospermia or severe oligospermia ($<5\times10^6$ spermatozoa/ml) (Franchimont *et al.*, 1972; de Kretser *et al.*, 1973; Bergmann *et al.*, 1994). The elevated FSH concentration is believed to be due to a decrease in inhibin secretion, which is produced by Sertoli cells (De Jong and Robertson, 1985). In cases of azoospermia with high FSH concentrations, a testicular biopsy is believed to be unnecessary (Hargreave and Jequier, 1978).

The aim of this article is to question all these assertions by discussing the regulation of FSH secretion in normal and infertile patients, by reviewing those situations where high FSH concentrations are associated with normal sperm production and by defining the usefulness of testicular biopsies in some cases of severe oligospermia or azoospermia associated with high serum FSH concentrations.

Physiological regulation of FSH secretion

FSH is released by the anterior pituitary gland under the influence of pulsatile secretions of gonadotrophin-releasing hormone (GnRH). The pituitary response to GnRH is determined by the frequency and amplitude of the GnRH pulses and the concentration of gonadal steroids (Finkelstein *et al.*, 1988). In men with hypogonadotrophic hypogonadism treated with GnRH, decreasing the pulse frequency of GnRH from one pulse/hour to one pulse every 3 h, induced an increase of FSH and of the FSH/luteinizing hormone (LH) ratio (Gross *et al.*, 1987). This increase may have been due to the slower metabolic clearance rate of FSH in comparison with LH (Coble *et al.*, 1969; Jockenhovel *et al.*, 1990). However, FSH concentrations remained in the normal range. The disproportionate rise in serum FSH compared with LH occurred only in GnRH-deficient men whose testosterone concentrations were in the hypogonadal range. It was not observed in gonadally mature patients. This was possibly due either to a normal concentration of sex steroids or to inhibin secretion by the mature gonad (Finkelstein *et al.*, 1988). Testosterone is the most important gonadal regulator of LH and FSH secretion. In normal and castrate men, graduated doses of testosterone produce a dose-dependent inhibition of LH and FSH because it exerts a negative feedback on the hypothalamus and the pituitary gland. Most of the gonadotrophin-inhibiting effects of testosterone can be accounted for by its aromatization into oestradiol (Snyder and Lawrence, 1980; Valk *et al.*, 1981; Matsumoto and Bremner, 1984; Finkelstein *et al.*, 1991).

FSH secretion is also regulated by two non-steroidal hormones, activin and inhibin, produced by Sertoli cells and which are also present in the pituitary (Ying, 1988). Inhibin and activin are dimeric glycoproteins sharing a common β-subunit. They have antagonistic actions on FSH secretion and have distinct paracrine actions on the gonads (Saez, 1994). Inhibin decreases FSH release whereas its secretion is stimulated by FSH and LH administration (McLachlan *et al.*, 1988, 1990). In contrast, activin increases the release of FSH (McLachlan *et al.*, 1989) and this effect is antagonized by follistatin (Meriggiola *et al.*, 1994). Follistatin is a glycoprotein which binds to activin and modulates its bioavailability (Moore *et al.*, 1994). In elderly men there is a decrease of basal and clomid-induced inhibin concentrations in comparison with young men, while serum FSH concentrations are higher in older men (Tenover *et al.*, 1988; MacNaughton *et al.*, 1991). The decrease in inhibin secretion may be related to a reduced number of Sertoli cells (Johnson *et al.*, 1984).

FSH increase in male infertility

In oligospermic men, serum FSH concentrations are usually normal when the sperm concentration is $>10\times10^6$/ml. FSH concentrations are usually above the normal range when sperm concentration is $<5\times10^6$/ml (de Kretser *et al.*, 1973; Wu *et al.*, 1981).

There are at least four possible mechanisms for the selective elevation of plasma FSH concentrations in men with a depressed sperm count: (i) the action of a hypothalamic FSH releasing hormone (activin) distinct from GnRH; (ii) an alteration in the pattern of GnRH pulses; (iii) a differential sensitivity of FSH and LH to sex steroid feed-back inhibition; (iv) inhibin deficiency.

Hypothalamic FSH release of hormone

Activin A has been shown to stimulate basal FSH and GnRH-stimulated FSH release in adult male primates (Ying, 1988). However, in the presence of inhibin, the stimulatory effect of activin is suppressed and it seems improbable that under these circumstances increased activin concentrations could account exclusively for FSH increase (Ying, 1988).

Altered patterns of GnRH pulses

A decrease in LH pulse frequency has been observed in some men with oligospermia and with increased FSH (slow pulsing oligospermia) (Wagner and Warsch, 1984; Gross *et al.*, 1985, 1986; Aulitzky *et al.*, 1989). In these men, pulsatile administra-

tion of GnRH every 2 h was able to decrease FSH and in some cases to increase sperm concentration (Wagner and Warsch, 1984; Gross *et al.*, 1986; Aulitzky *et al.*, 1989). However, these data have not been confirmed by other groups which observed a normal or an increased LH pulse frequency (Booth *et al.*, 1987; Wu *et al.*, 1989) and a lack of sperm improvement despite an FSH decrease (Bals-Pratsch *et al.*, 1989; Bremner *et al.*, 1993; Wu, 1993).

Several points must be clarified in order to explain these discrepancies: (i) what is the frequency of LH pulsatility in normal fertile men? Depending on the frequency of blood sampling (every 20 or 4 min), 10–30 pulses/24 h have been recorded (Veldhuis *et al.*, 1986). In studies measuring LH every 10 min, a wide range of pulse frequency was found in normal men, varying between 3 and 19 pulses/24 h (Spratt *et al.*, 1968; Veldhuis *et al.*, 1986). Therefore it is not possible to diagnose 'slow pulsing oligospermia' if blood is taken every 20 min as in Wagner's study (Wagner and Warsch, 1984); (ii) is FSH increase due to decreased LH frequency or to decreased testosterone concentrations? Even if there is an abnormal rise in serum FSH in GnRH deficient men, when the frequency of GnRH stimulation is decreased, such changes have not been found to elevate FSH above the normal range (Gross *et al.*, 1987; Spratt *et al.*, 1987). On the other hand, in men with primary testicular failure, an increased LH pulsatility has been observed (Matsumoto and Bremner, 1984). In conclusion, if FSH is still in the normal range a slight increase of the FSH/LH ratio could point to abnormalities of LH pulsatility, suggesting a moderate form of hypogonadotrophic hypogonadism which could be successfully treated by pulsatile GnRH administration (Spratt *et al.*, 1987). In cases with an abnormally raised FSH concentration, a primary testicular problem is almost certain and the efficiency of GnRH treatment remains to be proven.

Differential sensitivity of FSH and LH to steroid feedback inhibition

Differential sensitivity of FSH and LH to gonadal steroids is suspected. Different studies have shown that Leydig cell function may be altered in men with germinal epithelial injury and a selective increase in FSH concentration (de Kretser *et al.*, 1973; Ruder *et al.*, 1974; Gross *et al.*, 1987; Giagulli and Vermeulen, 1988; Tsatsoulis *et al.*, 1988; de Kretser *et al.*, 1989). In such patients, Booth *et al.* (1987) have shown that the production rate of testosterone was 50% of that in normal men but their oestradiol concentrations were normal. Therefore, the abnormal increase in plasma FSH relative to LH could result from small decreases in plasma testosterone concentrations. The negative feedback of testosterone is more pronounced on FSH than on LH (Valk *et al.*, 1981). In hypogonadal men treated with depot testosterone injections at 4 weekly intervals, an increase of FSH has been observed before a rise of LH when testosterone fell into the lower normal range (Snyder and Lawrence, 1980).

Inhibition deficiency

A deficiency of inhibin has been hypothesized. In comparison with the situation in older men, it has been postulated that a decrease of inhibin (due to a decreased Sertoli cell function) would be observed in cases of oligospermia or azoospermia. However, no (inverse) relationship between serum concentrations of inhibin and FSH has been established in four studies measuring inhibin in infertile men with azoospermia due to Klinefelter syndrome, idiopathic or chemotherapy induced seminiferous tubule damage (Tsatsoulis *et al.*, 1988; de Kretser *et al.*, 1989; Brennemann *et al.*, 1992; Vicari *et al.*, 1993). In most cases of azoospermia, serum concentrations of inhibin did not differ significantly from normal men. Different explanations have been given for these unexpected results: (i) inhibin immunoassays have been developed using antisera or antibodies raised to purified bovine inhibin or to synthetic N-terminal fragments of the α-chain. In all these assays, the free α-subunit, a non-bioactive molecule, cross-reacts with the dimeric molecule. Therefore the presence of circulating α-subunit related peptides secreted by the testis could limit the interpretation of the currently existing inhibin radioimmunoassay (Schneyer *et al.*, 1990); (ii) due to Leydig cell dysfunction in men with severe testicular damage, declining testosterone could cause an increase of FSH which in turn would stimulate the Sertoli cells to produce inhibin and maintain these normal concentrations as long as Sertoli cells are not altered by the pathologic process; (iii) the possible role of other FSH modulating substances, such as follistatin or activin, could also play a role (de Kretser *et al.*, 1989).

FSH increase with normal sperm production in old patients

Different studies have shown that daily sperm production declines significantly in older men (Johnson *et al.*,1984; Neaves *et al.*, 1984; Paniagua *et al.*, 1987). Although an inverse relationship has been observed between serum FSH and sperm production (Neaves *et al.*, 1984), moderate FSH concentrations in some older men may be able to maintain a normal sperm concentration. However, high serum FSH concentrations have been measured in men with involuted testes and reduced or arrested spermatogenesis (Paniagua *et al.*, 1987).

Unilateral orchidectomy

An increase in FSH and LH concentrations after unilateral orchidectomy for testicular cancer has been observed in several studies (Dunzendorfer and Weber, 1978; Fossa *et al.*, 1980; Willemse *et al.*, 1983). However, testicular cancer is frequently associated with infertility or with the secretion of human chorionic gonadotrophin (HCG) which can alter endogenous gonadotrophin concentrations (Berthelsen, 1984).

Increased FSH concentrations (3.7 ± 1.7 mIU/ml) were found in 22 patients who underwent unilateral orchidectomy for a benign disease, in comparison with a control group of 17 fertile men (1.5 ± 0.7 mIU/ml). FSH concentrations increased from 2.4 to 4.2 mIU/ml and LH concentrations from 2.7 ± 1.2 to 3.2 ± 1.3 mIU/ml when measured in seven patients before the operation and 10 days to 4 years after unilateral orchidectomy. There was no significant change in testosterone concentrations. Sperm analysis performed in 10 cases after unilateral orchidectomy revealed normal sperm concentrations ($>20\times10^6$/ml). In three cases with normal sperm count, FSH was increased to >6.5 mIU/ml (Mégevand *et al.*, 1985). These results suggest that compensatory FSH

increase after unilateral orchidectomy could be necessary to maintain a normal sperm production. This is comparable to other endorine situations such as an increase in thyroid stimulating hormone (TSH) in patients with partial thyroidectomy having normal thyroxine concentrations. The FSH increase after unilateral orchidectomy could be due to decreased inhibin concentrations according to studies performed in patients with germinal cancer or in animals (Schanbacher, 1988; Brennemann *et al.*, 1992).

Compensated spermatogenic failure

A few cases of increased serum FSH concentration associated with normal seminal parameters have been reported after mumps, orchitis, orchitis with an atrophic testis and after cryptorchidism suggesting, as after unilateral orchidectomy, that an elevated FSH concentration is compatible with relatively good sperm production and may not necessarily indicate severe germ cell failure (Wu *et al.*, 1981; Karpas *et al.*, 1983).

FSH-secreting pituitary adenoma

In all, 25–30% of pituitary tumours are considered as clinically nonfunctional (Oppenheim *et al.*, 1990). However, in four studies each involving 30–40 men (Snyder, 1985; Beckers *et al.*, 1985; Oppenheim *et al.*, 1990; Lahlou *et al.*, 1993), it was reported that 14–43% of all pituitary tumours involved a hypersecretion of FSH. The majority of these men with gonadotrophic adenoma did not oversecrete LH and had normal or subnormal testosterone concentrations (Snyder, 1985). Since their inhibin concentrations were increased, we must consider that their FSH was biologically active (Lahlou *et al.*, 1993). Sperm analysis, reported in only two of these cases, was normal (Woolf and Schenk, 1974; Berezin *et al.*, 1984). Most of the men with gonadotrophic cell adenoma were middle-aged men with a history of normal fertility. Examination showed that they were normally virile, with testes of normal size. The men had usually sought medical attention because of visual impairment due to the size of the adenoma (Snyder, 1985).

Utility of testis biopsy

In cases of azoospermia, associated with small testes and high FSH concentrations, a testis biopsy is usually considered unnecessary because FSH elevation is indicative of severe testicular damage such as Sertoli cell-only syndrome or early spermatogenic arrest (Hargreave and Jequier, 1978; Jarow *et al.*, 1989). However, we wish to discuss several exceptions to this rule.

Sertoli cell-only syndrome

This condition is characterized by the absence of germinal epithelium with the unique presence of Sertoli cells, small seminiferous tubules and small testes (Rothmann *et al.*, 1982). Among the 22 cases described by Rothman *et al.* (1982), eight had normal FSH concentrations and 14 elevated concentrations, suggesting the existence of two different types of Sertoli cell-only syndrome according to FSH concentrations. These data have been confirmed by several authors (Hargreave and Jequier, 1978; Bergmann *et al.*, 1994; Turek *et al.*, 1994). In the largest series, 88% (69 out of 78 patients) had elevated FSH concentrations (mean concentration was 3.2 times that of normal) and 12% (9 out of 78) had normal concentrations (Turek *et al.*, 1984). Although elevated FSH concentrations are considered to result from impaired Sertoli cell function, the precise nature of this dysfunction remains unknown. No relationship could be established between the morphological abnormalities of Sertoli cells and FSH concentrations (de Kretser *et al.*, 1981).

Spermatogenic arrest

This condition involves an interruption of the process of germ cell differentiation leading to the formation of spermatozoa resulting eventually in azoospermia. It has been observed in 4–30% of testicular biopsies in patients with azoospermia (Martin-du-Pan and Campana, 1993). There are conflicting data regarding which stage of spermatogenesis could be related to the inhibition of FSH production. Some investigators found an inverse relationship between serum FSH and the number of spermatogonia (de Kretser *et al.*, 1973; Martin-du Pan and Campana, 1993) but normal FSH concentrations in cases of blockade at the spermatocyte I stage. Other workers have found elevated FSH concentrations when spermatogenesis was blocked prior to the spermatid concentration (Franchimont *et al.*, 1972; Micic, 1983).

These discrepancies could be due to a decrease in the number of spermatogonia occurring in conjunction with a decreased number of spermatids (de Kretser *et al.*, 1973). On the other hand, the absence of late spermatids in rats was clearly associated with a marked decrease in the secretion of inhibin by seminiferous tubules (Allenby *et al.*, 1991). In man, the in-vitro production of inhibin by Sertoli cells was decreased when germ cells were absent (Foucault *et al.*, 1994). It is well-known that the various germ cells can specifically modulate different Sertoli cell functions, confirming the role of spermatids in the regulation of androgen-binding protein, FSH etc. (Jegou, 1993). From a practical point of view, in cases of azoospermia with a normal testicular volume and normal FSH concentrations, a testicular biopsy may be necessary to exclude spermatogenic arrest, except when immature sperm cells are present in the ejaculate.

Testis biopsy in azoospermic patients

The use of this procedure in azoospermic men with markedly elevated FSH concentrations is debated. In cases of azoospermia, if the serum FSH concentration is >three times normal and if the testes are atrophic, testicular biopsy is considered unnecessary (Hargrave and Jequier, 1978; Jarow *et al.*, 1989). In oligospermic patients, testicular biopsy could induce a decrease in sperm concentration (Rowley *et al.*, 1989). However, testicular biopsy has been advocated in cases of cryptorchidism in order to exclude a carcinoma *in situ*, since the risk of testicular cancer is increased four- to five-fold in that condition, the true prevalence being 2–3%. Of all patients with testicular cancer, 10% have a history of cryptorchidism (Giwercman *et al.*, 1989, 1993). However, infertility does not represent a severe risk, since in-situ carcinoma has been found only in 0.39% of 2043 infertile men and 0.6% of 2739 infertile men (Pryor *et al.*, 1983; Bettocchi *et al.*,

1994). Therefore it is difficult to justify a testicular biopsy for in-situ carcinoma.

Recently, gamete micromanipulation techniques using testicular sperm obtained by testicular biopsy have resulted in pregnancies (Schoysman *et al.*, 1993; Devroey *et al.*, 1994). In most of the cases, testicular biopsy was performed because of obstructive azoospermia with lack of spermatozoa in the epididymis. In that situation, serum FSH concentrations are normal and motile spermatozoa are usually found in the biopsy (Jow *et al.*, 1993). A recent study has shown that 48% (21 out of 44) azoospermic patients undergoing testicular biopsy and having FSH concentrations >30 mIU/ml had mature spermatozoa at the biopsy, whereas 52% (23 out of 44) had Sertoli cell-only syndrome (Gilbaugh *et al.*, 1994). If these data are confirmed, they would imply that spermatozoa could possibly be used for intracytoplasmatic sperm injection (ICSI). Therefore a testicular biopsy should be discussed and offered even to azoospermic patients with high FSH concentrations. This approach would require a genetic analysis of the patient since it has been found that 15% of azoospermic patients have abnormal karyotypes (Rivas, 1985).

Conclusion

In conclusion, this article tries to convince the reader that some current principles in male infertility should be reconsidered. Firstly, increased concentrations of FSH cannot be explained exclusively by a decreased inhibin or testosterone. Other (unknown) factors are probably involved. Secondly, an elevated FSH concentration does not always indicate a damaged germinal epithelium but may also reflect a compensatory adaptation to partial destruction or removal of testicular parenchyme, resulting in (sub)normal sperm production. Finally, testicular biopsies could be useful even in case of azoospermia with high FSH concentrations because if spermatozoa are found, they could be used for ICSI.

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Whither the investigation of male infertility? Is FSH measurement redundant and should all azoospermic patients have testicular biopsy?

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Recent reports of decreasing sperm counts and the increasing prevalence of developmental abnormalities in the male reproductive tract of Western men have raised the spectre of a trend of declining male fertility (Carlson *et al.*, 1992; Auger *et al.*, 1995). However, despite the explosion of basic information on testicular physiology in the last 20 years, the aetiologies and pathophysiology of defective spermatogenesis and spermiogenesis underlying the vast majority of patients suffering from male infertility remain poorly understood. Recent controlled trials on even relatively well-established 'rational' treatments for specific forms of male infertility such as immunosuppression for anti-sperm antibodies (Haas and Manganiello, 1987), ligation or embolization of varicoceles (Nieschlag *et al.*, 1994) and antibiotics for genital tract infections (Yanushpolsky *et al.*, 1995) have all proved disappointing. It is therefore exceptional for the clinician to be in a position to improve impaired semen quality. Consequently, assisted reproduction technologies (ART) have been increasingly applied to the empirical management of idiopathic male infertility, where a sufficient number of motile spermatozoa can be obtained, even though the results of treatment have remained relatively poor compared to that achievable in female infertility. Against this background, the astounding success recently reported with intracytoplasmic sperm injection (ICSI) using a single viable spermatozoon from severely oligozoospermic samples represent a notable beacon of hope for many patients previously considered beyond help (Van Steirtegheim *et al.*, 1993).

The review of Martin-de-Pan and Bischof (1995) is therefore important beyond the immediate topic addressed by the authors, namely the utility of follicle stimulating hormone (FSH) and testicular biospy in the investigation of the infertile male. It exemplifies the impact that micro-assisted reproduction technologies are exercising on our current and future approach to the management of male infertility. Since the establishment of radioimmunoassays, the measurement of FSH has become central to the assessment of the infertile male, especially in those with azoospermia. The main thrust of the review goes over familiar ground but with a new light which emphasizes the limitations of FSH measurement as an indirect index of seminiferous tubular failure. Thus normal FSH cannot distinguish between obstructive azoospermia and testicular germ cell arrest. Therefore testicular biopsy and/or microscopic examination of aspirated epididymal fluid is required. Until recently, the relatively poor results of conventional in-vitro fertilization (IVF) using spermatozoa obtained by micro-epididymal sperm aspiration (MESA) meant that it is not always appropriate to advise or proceed to an invasive investigation or expensive procedure for suspected obstructive azoospermia. However, with the considerably higher fertilization and implantation rates being reported with ICSI/MESA or the more simplified method of retrieval by percutaneous epididymal sperm aspiration (PESA) (Tsirigotis and Craft, 1995), the prospects for successful treatment in obstructive azoospermia have been considerably enhanced. A scrotal exploration and testicular biopsy would now be indicated in most patients where facilities for ICSI are available.

It has been widely accepted that persistent elevation of FSH concentration in the presence of azoospermia (especially associated with bilateral atrophic <10 ml testes) is synonymous with extensive and severe primary spermatogenetic failure, although a minority of seminiferous tubules may still retain some degree of germ cell development. Testicular biopsy under these circumstances was deemed unnecessary because of the lack of treatment and the poor prognosis. However, with the possibility of successful IVF by ICSI using spermatozoa retrieved from biopsied seminiferous tubules (Schoysman *et al.*, 1993; Devroey *et al.*, 1994) even in patients with atrophic testes and elevated FSH (Silber, 1995; Silber *et al.*, 1995a), the latter findings no longer condemn patients with non-obstructive azoospermia to the alternatives of donor insemination or adoption. It has been reported that nearly half (48%) of a group of azoospermic patients with FSH elevated to three times that of the upper limit of normal has mature spermatozoa at testicular biopsy (Gilbaugh *et al.*, 1994). This is consistent with the rethinking behind the diagnosis of Sertoli cell-only syndrome where it is now recognized that occasional foci of spermatozoa can be identified after careful search in more than half of such cases (Silber, 1995b). Recent reports of successful fertilization and implantation following intracytoplasmic injection of round spermatid nuclei (ROSNI) into rabbit (Sofikitis *et al.*, 1994) and human oocytes (Sofikitis *et al.*, 1995) potentially enhances the scope of treatment even further to include those patients with spermatogenic arrest, although it remains unclear whether the paternal centrosome was donated to the oocyte in this procedure. De Kretser's group also recently showed that hypergonadotrophic azoospermia does not rule out the presence of obstructive lesions and the possibility of fertility (Hauser *et al.*, 1995). The upshot of these considerations is that testicular biopsy should be offered to all azoospermic patients irrespective of FSH, clearly illustrating how established clinical practice is