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Is there a true requirement for follicle stimulating hormone in promoting spermatogenesis and fertility in primates?

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Although the role of follicle stimulating hormone (FSH) in regulating ovarian function is well accepted, its need in regulating testicular function of the adult continues to be debated. Sertoli cells of all mammals have FSH receptors and are known to regulate differentiation and transformation of germ cells to spermatozoa. However, there appear to be species and age differences in the way in which FSH regulates spermatogenesis. An attempt has been made in the current paper to examine critically the newer data available in support of and against the concept that FSH is required to regulate spermatogenesis and fertility of the primate. As there is no evidence to indicate that testicular function in monkeys and humans is regulated differently, the information available using the monkey as the experimental surrogate model is discussed in some depth. These are correlated, wherever feasible, to the newer information emerging from clinical studies. It appears from these studies that in the primate (including humans) FSH, besides promoting quantitative spermatogenesis leading to production of a normal number of spermatozoa, has a critical role in regulating spermiogenesis, the process that controls the formation of normal fertilizable mature spermatozoa. While the requirement for FSH in promoting fertility in the male monkey is reasonably well established, in humans the evidence currently available in favour of the concept is still circumstantial and more work needs to be done to establish the hypothesis beyond any doubt.

Key words: follicle stimulating hormone/primates/spermatogenesis

Introduction

The role of follicle stimulating hormone (FSH) in regulating ovarian function of a variety of animals, including primates, is clear and well defined. However, its role in regulating testicular function, in particular spermatogenesis, remains enigmatic. Recently two reports using a mouse FSH beta subunit knockout model (Rajendra Kumar *et al.*, 1997) and analysis of the infertile status of a group of homozygous men with an apparently inactivating mutation of the FSH receptor gene (566C→T) (Tapanainen *et al.*, 1997) concluded that FSH is not required for maintenance of spermatogenesis and fertility in humans. This in turn implies that anti-FSH based contraceptive vaccines are not a feasible proposition. In this short article, we wish to establish the reasons that make this conclusion untenable.

Non-primates

The requirement for a particular hormone to initiate and/or maintain spermatogenesis and fertility can be demonstrated in several ways other than through the use of gene knockouts. Besides the classical hypophysectomized rodent model used for 2–3 decades, investigators have used specific FSH antibodies in a rodent model to study similar events (Raj and Dym, 1976; Shivashankar *et al.*, 1977; Dym *et al.*, 1979; Vaishnav and Moudgal, 1994). These studies, using morphometry, flow cytometry and fertility testing as parameters, have shown that dependence on FSH for maintenance of spermatogenesis can more clearly be demonstrated in the immature rather than in the adult rat. The flow cytometric (FCM) analysis of germ cells revealed that transformation of spermatogonia (diploid, 2N) to primary spermatocytes (4N) and round spermatids (1N) as well as the overall conversion of diploid (2N) to haploid (1N) spermatogonia is reduced by >90% in the FSH-deprived immature rat testis and by 54 and 26% respectively in the FSH-deprived adult. The ratio of elongating/elongated (haploid) spermatids to diploid spermatogonia, however, was reduced by 78% in the FSH deprived adult. In addition, the residual serum FSH concentration (reciprocal of antibody titre) was correlated to haploid and 4N germ cell populations, providing clear evidence for FSH involvement in spermiogenesis (Vaishnav and Moudgal, 1994). Germinal cell types in rat testis were significantly reduced following immunization with gonadotrophin-releasing hormone (GnRH), while recombinant FSH therapy partially restored spermatogenesis, leading to an increase in the number of spermatogonia and germ cells at subsequent maturational stages (Mclachlan *et al.*, 1995). The need for FSH to maintain spermatogenesis in the hamster (Lerchl *et al.*, 1993) and sheep (Kilgour *et al.*, 1993) has also been noted. Using gonadotrophin deficient (hpg) mice, Singh *et al.* (1995) observed that testosterone and dihydrotestosterone (DHT) could initiate qualitatively complete spermatogenesis in the mouse, leading to the production of fertile spermatozoa despite low intratesticular androgen levels and the absence of blood FSH. Even in this situation, the number of germ cells per Sertoli cell was reduced. To a large degree, the data of Rajendra Kumar *et al.* (1997) obtained from FSH beta gene knockout mice essentially confirm the earlier data. Their report

suggests that data obtained from the mouse should pertain to all mammals, including humans, but this clearly is not the case. There appears to be sufficient evidence to show that there are distinct differences in the way hormones (particularly FSH) regulate spermatogenesis in the adult rodent and the primate (Zirkin *et al.*, 1994). Even in mice, when FSH signalling is perturbed by other means, the majority of spermatozoa produced are abnormal (Sairam, unpublished results), with a consequent reduction in fertility. Compensating mechanisms may come into effect in genetically altered mice, and extrapolation across the species barrier is hazardous. Of particular interest in this context is the observation of Accili (1997) regarding the use of insulin receptor gene knock out mice to study insulin action. He noted that while this model is useful in determining gene function, the conclusions reached are applicable to mice and not to humans.

Primates

The study of Matsumoto *et al.* (1986) has elegantly shown that blocking endogenous FSH secretion in normal men leads to significant inhibition in sperm output which can be reversed by exogenous FSH but not by testosterone supplementation. Several clinical studies have observed that exogenous supplementation with pure FSH (including recombinant material) along with human chorionic gonadotrophin (HCG) is needed to initiate as well as promote quantitative spermatogenesis and fertility in hypogonadotrophic hypogonadal men (Acosta *et al.*, 1992; Kliesch *et al.*, 1995; Gromoll *et al.*, 1996; Anderson *et al.*, 1997; Burgues and Calderon, 1997).

Since there is no concrete evidence to suggest that hormonal regulation of spermatogenesis in the monkey is different from that of the human, this species has been extensively used as a human surrogate model. Studies in the non-human primate carried out by our group (Murthy *et al.*, 1979; Moudgal, 1981; Moudgal *et al.*, 1992; Aravindan *et al.*, 1993) and others (Wickings *et al.*, 1980; Raj *et al.*, 1982, 1991; Srivatsava and Das, 1992) have clearly shown that deprivation of endogenous FSH support obtained using an immunological approach results in arrest of spermatogenesis. Srinath *et al.* (1983) showed that arrest in monkey spermatogenesis achieved by immunizing them with oFSH for a 2 year period was partially reversed by continuing immunization for a 4.5 year period using repeat boosting of reasonably high quantities of bioactive FSH. This has unfortunately been wrongly interpreted as evidence for the non-involvement of FSH in spermatogenesis. That the partial recovery could be the result of an immunological sequela rather than demonstrating the lack of FSH effect is overlooked. Using FCM analysis, Aravindan *et al.* (1993) have offered support for our hypothesis that FSH deprivation in monkeys leads to inhibition of spermatogonial proliferation and transformation to primary spermatocytes. Evidence for an impairment in the spermiogenic process following FSH deprivation has also been provided by Aravindan (1991, 1997). The low number of spermatozoa produced under these circumstances has been shown to be of poor functional quality leading to infertility (Moudgal *et al.*, 1992).

Theoretically, it should also be possible to block FSH function with an appropriate and specific FSH receptor antibody. This has been achieved using a recombinant FSH receptor protein fragment (oFSHR-P) as the immunogen in adult male monkeys. The end results (arrest in spermatogenesis, production of low numbers of poor quality spermatozoa and infertility) were essentially similar to those obtained following oFSH immunization. In both cases serum testosterone concentrations remained unchanged (Moudgal *et al.*, 1997a). It has been shown that Sertoli cell function of both normal adult monkey and man is significantly affected by specific deprivation of FSH support (Moudgal *et al.*, 1997b). Confirmation that FSH influences Sertoli cell function in adult men is available from the work of Anderson *et al.* (1997). More importantly the spermatozoa of both FSH immunized monkeys and men as well as FSH receptor immunized monkeys exhibited similar characteristics: a marked reduction in acrosomal glycoprotein content and defective chromatin packaging (Krishnamurthy *et al.*, unpublished data). Both the above parameters are known to be associated with human male infertility (Cross *et al.*, 1986; Evenson and Jost, 1994).

In the light of these studies, the observation of Tapanainen *et al.* (1997), based on the analysis of homozygous males with an apparently inactivating mutation of FSH receptor gene (566C→T predicting an alanine to valine change of amino acid), that FSH may not have an essential role in normal human male fertility is intriguing. An analysis of their data shows that three out of five men examined exhibited a 51–73% reduction in testicular volume and a >2.0 fold increase in circulating FSH and luteinizing hormone (LH) levels. One of the men was infertile, while the fertility status of the other two was not known. In contrast, the two fertile men had near normal testicular size and normal FSH and LH levels. It would have been pertinent if, in addition to fertility, paternity had also been established. The possibility exists that the FSH receptor gene was not mutated in the same way in all five cases. No direct evidence was provided to suggest that the mutated FSH-R bound FSH only marginally. In contrast, a recent clinical report by Aittomaki *et al.* (1996) indicated that ovarian dysgenesis observed in a group of women exhibiting receptor mutation 566C→T was pathogenetically distinct from the group exhibiting ovarian dysgenesis without receptor mutation. The presence of follicles in the mutated receptor group was indicative, according to the authors, of the presence of residual receptor activity. It is possible that two of the homozygous fertile men examined by Tapanainen *et al.* (1997) bearing a similar FSH-R mutation also exhibited residual receptor activity. Particularly in the case of the male, a weakly active FSH receptor in the presence of elevated concentrations of the hormone may provide a tonic stimulus adequate for maintaining spermatogenesis. Interestingly, none of the men examined appeared to have normal sperm parameters, most being acutely oligospermic. When spermatozoa were present, they were not normal, very similar to the situation occurring when monkeys were deprived of FSH support by immunoneutralization with oFSH (Aravindan *et al.*, 1991; Moudgal *et al.*, 1992). Based on the results of a study of hypophysectomized men with an activating mutation of FSH receptor (Gromoll *et al.*, 1996), it has been suggested that FSH can sustain sperm

production in men permanently, even in the absence of adequate concentrations of intratesticular testosterone.

The data available from monkey studies (Aravindan *et al.*, 1991, 1993, 1997) as well as the limited amount of information from the human studies indicates that FSH deprivation affects spermiogenesis and hence the functional quality – chromatin packaging, reduced acrosomal glycoprotein concentration (Krishnamurthy *et al*., unpublished data), inability to fertilize zona denuded hamster eggs (Sharma and Das, 1992) – of ejaculated spermatozoa. Oligospermia coupled with poor sperm quality is a major cause of infertility in men. The work of Acosta *et al.* (1992), Bartoov *et al.* (1994) and Kleish *et al.* (1995) indicated that pure FSH therapy without or with HCG in a group of infertile/subfertile men significantly enhanced sperm concentration and quality, leading to improved fertilization. The studies of Baccetti *et al.* (1997) showed that FSH therapy in a group of responsive infertile men improved sperm quality towards the natural fertility threshold level, particularly certain qualities of the acrosome, chromatin, mitochondria and the xenome. These studies, by suggesting that FSH has a marked effect on spermiogenesis, support our finding that FSH deprivation in the fertile monkey/human male does effect sperm concentration and quality. In the light of the variety of studies described above it does not appear justified for Tapanainen *et al.* (1997) to comment about fertility without carrying out an unbiased test for in-vitro fertilizing capability of individual sperm samples of all subjects using either human or zona denuded hamster eggs. The spermatozoa of these individuals should also be checked for defective chromatin packaging, acrosome content etc. as FSH deprivation in both man and monkey has been shown to affect these parameters.

In conclusion, although naturally occurring or artificially created mutational changes in FSH or FSH receptor gene in animals or humans provide a novel means of studying the requirement for a hormone to maintain a key physiological event, it is not possible to use this approach to reach a decisive conclusion regarding hormonal need. Generally, perturbation of FSH signalling in the testis leads to aberrant gametogenesis and FSH lack has been suggested to result in apoptosis of not only spermatogonia and primary spermatocytes but also of spermatids/spermatozoa. The consequences of FSH deficiency, however, have been shown to vary in different species. Hitherto, no evidence is available to indicate that spermatogenesis/testicular function in monkeys and men are regulated differently. Consequently the data obtained in non-human primates using a specific immunological procedure to bioneutralize FSH or to block FSH receptor function cannot be ignored. The data available from the study of the human male, though preliminary in nature, clearly point to essential similarity between men and monkeys in their response to blockade of FSH function. A fair amount of clinical data hitherto available is supportive of FSH having a significant role in promoting quantitative spermatogenesis, leading to the sperm quality needed for successful fertilization. In view of this, we believe that without more critical experiment/evaluation of clinical data on receptor mutations, it is inappropriate at this time to conclude that FSH has no role in promoting human male fertility. We propose that the reduction in sperm counts accompanied by increase in abnormalities, observed under conditions of FSH deprivation, produces a condition equivalent to oligozoospermia and teratozoospermia, believed to be a common cause of infertility in men.

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