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Responsiveness of human male volunteers to immunization with ovine follicle stimulating hormone vaccine: results of a pilot study

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A study of 140 days duration was performed to examine if human male volunteers ($n = 5$) respond to ovine follicle stimulating hormone (oFSH) immunization (administered adsorbed on Alugel on days 1, 20, 40 and 70) by producing antibodies capable of both binding and neutralizing bioactivity of human FSH. The kinetics of antibody production for both the immunogen (oFSH) and the cross-reactive antigen (hFSH) were essentially similar. The volunteers responded only to the first two immunizations. The boosters given on days 40 and 70 were ineffective, probably because of the presence of substantial amounts of circulating antibody to oFSH. Of the antibodies generated to oFSH, 25–45% bound hFSH with a mean binding affinity of $0.65 \times 10^9 \pm 0.53$ M^{-1}. The binding capacities at the time of high (30–80 days of immunization) and low (>110 days) titres were 346 ± 185 and 10.5 ± 5.8 ng hFSH/ml respectively. During the period of high titre, free serum FSH (value in normal males 1–5 ng/ml) was not monitorable. A 50 µl aliquot of the antiserum obtained from different volunteers between days 30 and 80 and on day 140 blocked binding of ^{125}I-labelled hFSH to its receptor by 82 ± 9.7 and 53 ± 12.2% respectively. The antibody produced was specific for FSH, and no significant change in the values of related glycoprotein hormones (luteinizing hormone/testosterone and thyroid stimulating hormone/thyroxine) were recorded. Seminal plasma transferrin, a marker of Sertoli cell as well as of seminiferous tubular function, showed marked reduction (30–90%) following immunization with oFSH. Considering that endogenous FSH remained neutralized for approximately one sperm cycle only (65 days), the reduction in sperm counts (30–74%) exhibited by some volunteers is encouraging. Immunization with oFSH did not result in any significant changes in haematology, serum biochemistry or hormonal profiles. There was no production of antibodies capable of interacting with non-specific tissues. It is concluded that it should be possible to obtain a sustained long-term blockade of endogenous FSH action in men by using oFSH as an immunogen. This is a prerequisite for obtaining significant reduction in the quality and quantity of spermatozoa produced, thus leading to infertility.

Key words: FSH antibody in man/FSH vaccine/seminal transferrin/Sertoli cell

Introduction

There appears to be a clear consensus on the need for follicle stimulating hormone (FSH) in maintaining quantitative spermatogenesis in the monkey (Sheela Rani *et al.*, 1978; Murty *et al.*, 1979; Wickings *et al.*, 1980; Moudgal, 1981; Raj *et al.*, 1982), human (Matsumoto *et al.*, 1986; Gromoll *et al.*, 1996) and hamster (Lerchl *et al.*, 1993). However, there is evidence both for and against the need for FSH in regulating spermatogenesis in the adult rat (Dym *et al.*, 1979; Vaishnav and Moudgal, 1991, 1994; Shetty *et al.*, 1996) and mice (Singh *et al.*, 1995).

Earlier work from our laboratory has shown that adult male fertile bonnet monkeys immunized with ovine FSH (oFSH) generate antibodies capable of binding monkey as well as human FSH (hFSH; Moudgal *et al.*, 1992). A consequence of bioneutralization of endogenous FSH by the circulating antibody is disruption in spermatogenesis, leading to oligospermia and infertility (Moudgal *et al.*, 1992; Aravindan *et al.*, 1993). Adopting such an approach for male contraception appeared attractive, as it had no effect on serum testosterone and libido, a problem that besets other steroid-based male contraceptive procedures currently under test. Extensive pre-clinical toxicology studies carried out in the rat (by N.Sethi, CDRI, Lucknow, India) and the monkey (by S.Sehgal, PGIMER, Chandigarh, India) have revealed that the oFSH vaccine at the doses tested (1× and 5× proposed human dose) was not harmful and was free from toxicity (Sehgal *et al.*, 1991). We report in this paper the results of a pilot study on immunization of five human male volunteers with the oFSH vaccine. The objectives of this study were twofold: to determine whether oFSH administered adsorbed on Alugel (a relatively mild adjuvant cleared for human use) elicits an immune response in the human male similar to that in the monkey, and whether oFSH immunization carried out thus is likely to be harmless and non-toxic to the human.

Materials and methods

The vaccine

The oFSH used as the immunogen was isolated in the laboratory using conventional purification procedures (Moudgal *et al.*, 1992) from

freeze-dried sheep pituitaries imported from Waitaki International Biosciences (Christ Church, New Zealand). Pituitaries from this source were preferred as sheep in New Zealand are certified free of schedule-1 diseases such as foot and mouth, rinderpest, vesicular stomatitis, anthrax, swine fever, lumpy skin disease, contagious bovine pleuropneumonia and scrapie. The vaccine grade oFSH was also characterized for purity by electrophoretic procedures using appropriate radioimmuno- and radioreceptor assays. The purified preparation (batch no. 4) was tested for the presence of mycoplasma, as well as haemadsorbing and non-adsorbing viruses and other extraneous non-specific disease-causing agents by the Serum Institute (Poona, India), a laboratory which routinely undertakes quality control tests using World Health Organization (WHO, 1988) guidelines on vaccines produced for use in humans. The adjuvant used was Alugel (aluminium hydroxide gel; Superfos, Vedbaek, Denmark), and the vaccine formulation using pyrogen-free distilled water as well as the ampouling itself were performed using the GMP facility of Karnataka Antibiotics Ltd. (Bangalore, India). Care was taken to prevent cross-contamination by ampouling each of the doses separately.

Selection of volunteers

The volunteers chosen were normal healthy men aged 34–41 years who had completed their families. Besides screening them for normal health, the sera of individual volunteers were tested prior to their inclusion in the study for the absence of autoantibodies to mitochondria, smooth muscle, nuclear, thyroid microsomal, thyroglobulin, parietal cell, adrenal, pancreas and pituitary. They were also tested for the absence of C-reactive protein, rheumatoid factor and human immunovirus (HIV). Out of 14 volunteers screened, the five who passed all tests were recruited to the study.

Approval for the study

This study was approved by the Indian Council of Medical Research, New Delhi, the Drug Controller, Government of India and the Ethics Committee for safe human experimentation of the Ramaiah Medical College Hospital, Bangalore. Each of the volunteers provided signed letters of informed consent.

Vaccination protocol

Each of the volunteers received s.c. 1.0 ml of the gel suspension containing 1 mg of oFSH on day 1 and on day 20 a further 1 ml of suspension containing 0.3 mg of oFSH. Booster doses of 0.1 mg of oFSH were administered on days 40 and 70 of immunization. Each of the above injections was preceded by a test dose of 0.1 ml given intradermally 30 min prior to the main dose. The dose schedule used here was primarily based on data obtained from earlier studies in monkeys (Moudgal *et al.*, 1992).

General methodology

Blood samples (5–10 ml) were obtained just prior to each of the injections as well as on other days as indicated in the Results section. Haematological and serum biochemical analysis were performed using a Coulter counter (Transasia model sysmex K-1000, Hialeah, FL, USA) and an autoanalyser (model 550 express; Ciba Corning Diagnostic Corporation, Oberlin, New York, USA) respectively. Serum hormone concentrations [luteinizing hormone (LH), testosterone, thyroid-stimulating hormone (TSH), thyroxine, triiodothyronine, prolactin and cortisol] were determined using IRMA Coat-A-Count radioimmunoassay kits (Diagnostic Product Corporation, Los Angeles, CA, USA).

Antibody titration was performed using ^{125}I-labelled hFSH (kindly provided by NIAMDD, Bethesda, MD, USA and M.R.Sairam, Clinical Research Institute, Montreal, Canada) and oFSH (laboratory preparation) by methods described earlier (Moudgal *et al.*, 1992). To determine the extent of cross-reactivity of the oFSH antibody with hFSH, 1:100 diluted serum samples from individual human volunteers were incubated in the presence and absence of hFSH (1 μg/tube) overnight at room temperature followed by addition of ^{125}I-labelled oFSH (150 000 cpm/tube) and a further incubation for 5–6 h. Next, the antigen–antibody complex in solution was precipitated by adding polyethylene glycol (PEG 6000); the pellet was monitored after centrifugation and washing for radioactivity. Percentage reactivity was calculated by assuming binding of ^{125}I-labelled oFSH to the antibody in the absence of hFSH to be 100%. The bioneutralizing activity of the antisera was determined using a sheep testicular FSH receptor binding inhibition assay as described earlier (Aravindan *et al.*, 1993). The presence, if any, of unreacted 'free FSH' in the sera of immunized volunteers was determined using a two-step radioreceptor assay system standardized in the laboratory. In this assay, the sera of volunteers (100 μl) were incubated with a fixed aliquot of a rat testicular receptor preparation (a 30 000 *g* pellet) for 2 h at room temperature followed by centrifugation and washing. The resuspended receptor pellet was then incubated with ^{125}I-labelled hFSH for 1 h at room temperature, followed by washing and monitoring of receptor-bound radioactivity. A standard curve was constructed by substituting volunteer sera with different aliquots of a hFSH solution of known concentration. The range of the assay was from 0.3 to 25 ng hFSH/tube.

To determine whether immunization with oFSH evoked production of antibodies to non-specific tissues, the reaction of sera with processed tissue obtained from fresh autopsied organs was assessed using indirect immunofluorescence. Briefly, all sera were diluted 1:10 and allowed to react with different tissue substrates for 30 min at 37°C. After extensive washing, the sections were treated with an appropriate fluorescein-conjugated anti-human immunoglobulin G. The sections were mounted in buffered glycerol and viewed in a Zeiss fluorescent microscope and the intensity of reaction was arbitrarily graded from – to + to ++++. An enzyme-linked immunosorbent assay was used for HIV and the latex test to detect rheumatoid factor.

Semen obtained periodically by masturbation was analysed for sperm counts, viability and gross motility of spermatozoa according to the WHO (1992) manual. The sperm-free seminal plasma was stored below 4°C until used for the measurement of transferrin concentration by an appropriate radioimmunoassay. Human recombinant transferrin and goat antiserum to transferrin were purchased from Cappel (Westchester, PA, USA).

Results

All the five volunteers maintained normal health over the entire period of 140 days (treatment for 70 days and post-treatment 70 days). Allergy or hypersensitivity disorders were not detectable in any of the subjects. There was no adverse immediate reaction to immunization, though slight swelling over the site of injection on days 1, 20, 40 and 70 was discernible even 10 days post-injection. The volunteers did not complain of discomfort and the swellings disappeared with time. No appreciable changes in either body weight or testicular size of the individual subjects were recorded.

Haematology, serum biochemistry and hormonal profile of immunized volunteers

Blood samples of individual volunteers were analysed during the pretreatment (on days –7 and 0), treatment (on days 10,

Table I. Serum hormone profile in male volunteers immunized with ovine follicle stimulating hormone. Mean ± SD (n = 5) was calculated for serum concentration on days of immunization, pretreatment days –7 and –10, treatment phase days 20, 30, 40, 50, 60 and 70 and post-treatment phase days 80, 110, 140 and 170

Hormones	Normal range	Pre-treatment period	Treatment period	Post-treatment period
LH (mIU/ml)	0.4–3.7	1.1 ± 0.66	1.26 ± 0.8	1.37 ± 0.94
TSH (mIU/ml)	0.3–5.0	1.1 ± 0.5	1.3 ± 0.85	2.2 ± 1.05
Testosterone (ng/dl)	270–1070	683 ± 93.5	677 ± 109	656 ± 116
Thyroxine (μg/dl)	4.5–12.5	5.5 ± 0.56	5.36 ± 0.5	5.75 ± 0.35
Triiodothyroxine (ng/dl)	86–187	113 ± 34	124 ± 31	100 ± 0
Cortisol (μg/dl)	5–25	9.3 ± 3.0	10.3 ± 2.7	9.75 ± 4.6
Prolactin (ng/ml)	5–20	5.9 ± 1.5	6.1 ± 0.85	6.6 ± 1.5

Table II. Screening of sera of human volunteers immunized with ovine follicle stimulating hormone for non-specific autoantibodies[a]. The intensity of antibody staining on the tissue was given an arbitrary score of – to ++++

Volunteer no.	Day of sampling	ANA	SMA	PCA	AMA	PIT	ADR	PAN	THYR	CRP	LAT
1	1	–	–	–	–	–	–	–	–	–	–
	70	–	–	–	–	–	–	–	–	–	–
	140	–	–	–	–	–	–	–	–	–	–
5	1	–	±	–	–	–	–	–	–	+	+
	70	–	–	–	–	–	–	–	–	+	+
	140	–	–	–	–	–	–	–	–	–	–
9	1	–	–	+	–	–	–	–	–	–	–
	70	–	–	+	–	–	–	–	–	+	+
	140	–	–	+	–	–	–	–	–	+	++
10	1	–	–	–	–	–	–	–	–	+	++
	70	–	–	–	–	–	–	–	–	–	–
	140	–	–	–	–	–	–	–	–	–	–
12	1	–	–	–	–	–	–	–	–	–	–
	70	–	–	–	–	–	–	–	–	–	–
	140	–	–	–	–	–	–	–	–	–	–

[a]ANA = antinuclear; SMA = antismooth muscle; PCA = parietal cell; AMA = antimitochondrial; PIT = antipituitary; ADR = antiadrenal; PAN = antipancrease; THYR = antithyroid; CRP = C-reactive protein; LAT = latex test for rheumatoid factor.

20, 30, 40, 50 and 70) and post-treatment (on days 80, 100 and 140 days) phases for a variety of parameters. The haematological parameters analysed included haemoglobin, total lymphocyte count, percentage of neutrophils, lymphocytes, eosinophils, packed cell volume and erythrocyte sedimentation rate (ESR). All of these parameters remained within the normal range for the duration of the study. The serum biochemistry carried out included analysis for fasting blood sugar, urea, creatinine, triglyceride, high-density lipoprotein, very-low-density lipoprotein, low-density lipoprotein, serum glutamic−oxalacetic transaminase and serum glutamic−pyruvic transaminase. Values for all volunteers for the duration of the study were within the normal range. The serum concentrations of LH, testosterone, TSH, triiodothyronine, thyroxine, cortisol and prolactin analysed at different phases of immunization were also within the normal range for the entire study period (Table I).

Immunopathological screening of sera of volunteers at different stages of oFSH immunization

Sera of individual volunteers obtained on days 1, 70 and 140 of immunization were analysed for the presence of autoantibodies to a variety of tissue-specific antigens (Table II). None of the patients exhibited significant antibody titres to thyroid microsomes or adrenal and pancreatic islet cells. While one sample showed faint smooth muscle antibodies, in another case faint antibodies to parietal cells were detected. During immunization, sera from three volunteers exhibited increased amounts of rheumatoid factor and C-reactive protein activity, but these disappeared in two of these volunteers by day 140.

Titration of antibodies to ovine and human FSH

The relative antibody concentration as a function of time for both oFSH and hFSH showed a similar pattern, maximal activity being exhibited between days 30 and 80 of immunization. The antibody titre started to fall by day 110 and reached minimal values beyond day 140 of immunization (Figure 1). Except for volunteer 1, who showed a slightly delayed response, the volunteers exhibited significant increments in oFSH antibody titres by day 30 and maximal values were maintained through day 80. The antibody titres to hFSH relative to the immunogen (oFSH) were substantially low. Since a proportion of the cross-reactive antibody produced would have been neutralized by endogenous circulating hFSH, the hFSH antibody titre determined represents only the excess 'free' antibody pool. It appears that all of the human volunteers responded only to the first two injections of oFSH, given on days 1 and 20, but not to the boosters of 100 μg oFSH given on days 40 and 70. The cross-reactivity of the oFSH antibody with hFSH was measured by determining binding to ^{125}I-labelled oFSH

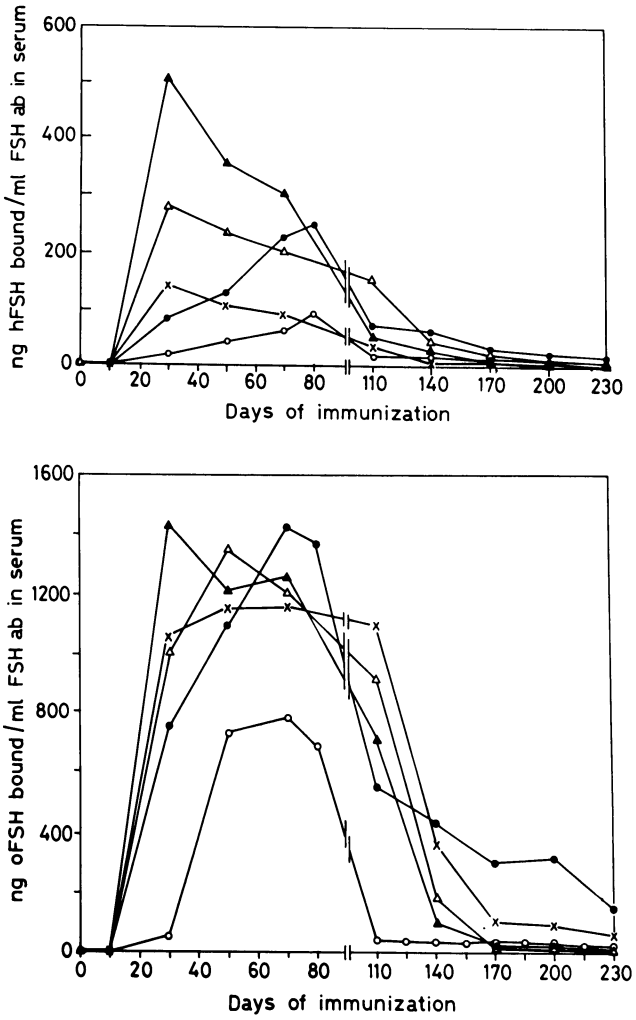

Figure 1. Antibody titration with ^{125}I-labelled ovine (upper panel) and human (lower panel) follicle stimulating hormone (FSH). A fixed aliquot of serum samples from human volunteers was incubated with ^{125}I-labelled hFSH, upper panel, or oFSH, lower panel, overnight at room temperature, following which the mixture was subjected to polyethylene glycol (PEG 6000) precipitation, centrifuged at 1600 *g* and the radioactivity in the pellet monitored in an LKB Clinigamma. Values represented are means of duplicate samples. Volunteer number 1, ○; 5, ●; 9, Δ; 10, ▲; 12, ×.

in the presence and absence of excess amount of hFSH, and this ranged between 26 and 45%.

Characterization of antibodies cross-reactive with hFSH

The antibodies cross-reactive with hFSH exhibited high binding affinity (0.3×10^9–1.6×10^9 M^{-1}). The binding capacity was maximal on days when antibody titre was high (between days 30 and 80) and reached minimal values by day 140, when the antibody titre was low (Table III). Considering that the mean FSH concentration in normal human males is between 3 and 15 mIU/ml (1–5 ng/ml), the antibody titre between days 30 and 80 of immunization should be more than adequate to neutralize endogenous FSH.

The bioneutralizing capacity of the antibody was determined by pre-incubating ^{125}I-labelled hFSH with sera samples of volunteers prior to incubation with a particulate sheep testicular receptor preparation (Table III). Sera from individual volunteers showed differences in their ability to inhibit binding of ^{125}I-labelled hFSH to the receptor. This ability was maximal (82 ± 9.7%) when the antibody titre was high, and when the titre fell to low values (by day 140) the % inhibition was also reduced (53 ± 12.2%). When the antibody titre was relatively high, free FSH (not bound to the antibody) in the sera was undetectable by the two-step radioreceptor assay employed (Figure 2).

Semen characteristics and seminal plasma transferrin

The semen samples collected from individual volunteers during the pre-treatment, treatment and post-treatment phase were analysed for normality according to the WHO (1992) protocol. No marked changes were observed in the motility or viability (range 40–60%) of the ejaculated spermatozoa. No significant morphological abnormalities were noticeable. Sperm counts obtained on days –7 and 0 of immunization (considered as control) were compared to those obtained on days 50 and 70 (representing treatment phase) and days 100 and 140 (representing post-treatment phase) of immunization. Of the five volunteers, four showed reduction of ~33%, while one volunteer (no. 9) showed a 64% reduction in sperm count (Table IV). As the effect of immunization is generally extended into the early post-treatment phase, it is not surprising that some volunteers showed reduced sperm counts even between days 110 and 140.

Seminal plasma transferrin was measured using a human transferrin radioimmunoassay system. Using a recombinant human transferrin preparation as a standard, the assay was able to measure within the range 5–100 ng/tube. Individual volunteers showed marked reduction (in the range of 30–90%) in seminal plasma transferrin compared with normal values. The % reduction observed varied as a function of days of immunization (Figure 3).

Discussion

The current study clearly establishes that antibodies produced in the human male to oFSH are able to recognize and bind hFSH with great avidity. From the profile of the antibody titre to ovine and human FSH it can be inferred that the immunological response to epitopes in these two proteins is essentially similar. This is not surprising, as considerable homology exists between the β subunits of ovine and human FSH (Ward and Bousfield, 1990). All five volunteers responded to the immunogen, peak antibody titres being obtained in three volunteers (nos. 9, 10 and 12) as early as day 30–50 and in two (nos. 1 and 5) between days 70 and 80 of immunization. Whereas the titre of the antibody population cross-reactive with hFSH was markedly reduced by day 110, that reactive with oFSH reached low values only by day 140 and beyond. The reason why the volunteers did not respond to the booster injections given on days 40 and 70 was sought by examining the resting antibody concentrations to oFSH at the time the boosters were given. The antibody titres to oFSH between days 50 and 70 were still very high (binding capacity 1140 ± 110 ng oFSH/ml), and appeared more than adequate to neutralize the booster dose given. Based on total blood volume of

Table III. Characteristics of the human follicle stimulating hormone (hFSH) cross-reactive antibody

Volunteer no.	Day of immunization	Binding affinity (M^{-1})	Binding capacity[a] (ng hFSH/50 µl serum)	Bioneutralization activity[b] (% inhibition/50 µl serum)
1	80	0.4×10^9	3.75	73
	140	0.4×10^9	0.3	42
5	80	1.6×10^9	12.5	74
	140	1.6×10^9	3.0	55
9	30	0.4×10^9	14.5	95
	140	0.4×10^9	2.3	71
10	30	0.56×10^9	25.0	78
	140	0.56×10^9	0.6	41
12	30	0.3×10^9	7.3	89
	140	0.3×10^9	1.2	54

[a]Based on the results (direct binding of the hormone ligand to the antibody) provided in Figure 1.
[b]Receptor binding inhibition was determined by pre-incubating ^{125}I-labelled hFSH (~200 000 cpm/tube) with 50 µl of volunteer serum overnight at 4°C prior to addition of the sheep testicular receptor preparation and continuing incubation for 60 min at room temperature. Polyethylene glycol (PEG 6000) was added to a final concentration of 5% to facilitate completion of precipitation. Use of 50 µl of normal human serum inhibited binding of ^{125}I-labelled hFSH to the receptor by only 10–15%.

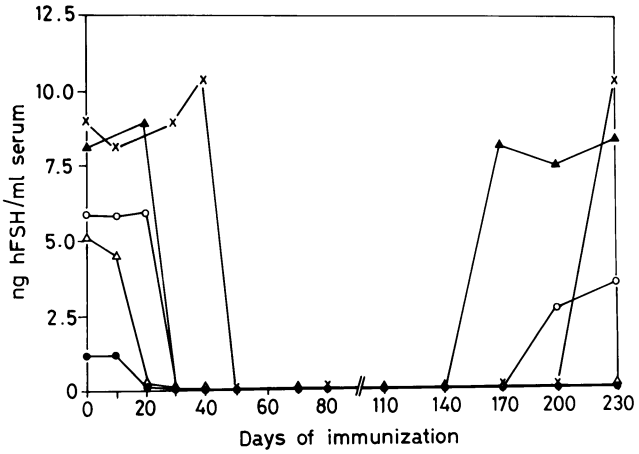

Figure 2. Measurement of 'free follicle stimulating hormone' in sera of immunized human volunteers. For details of methodology see text. Volunteer number 1, ○; 5, ●; 9, △; 10, ▲; 12, ×.

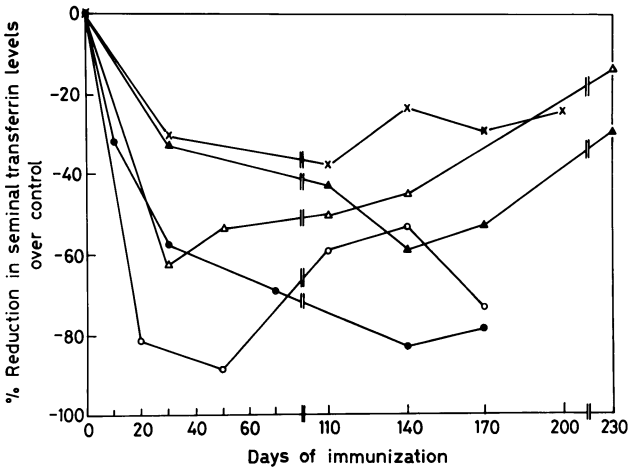

Figure 3. Measurement of transferrin in the seminal plasma of immunized human volunteers by radioimmunoassay. The procedural details are provided in the text. An aliquot of 50–100 µl of seminal plasma was used for each assay. The seminal plasma transferrin value of normal human males was 56.3 ± 7.8 µg/ml. Volunteer number 1, ○; 5, ●; 9, △; 10, ▲; 12, ×.

Table IV. Sperm counts ($\times 10^6$/ml) and percentage motility of follicle stimulating hormone-immunized volunteers. The values are means of sperm counts in ejaculates obtained on days –7 and 0 during pretreatment, days 50 and 70 of treatment and days 110 and 140 of post-treatment. The numbers in parentheses represent the percentage of motile spermatozoa

Volunteer no.	Pre-treatment phase	Treatment phase	Post-treatment phase
1	44.8 (55)	23 (40)	22.5 (45)
5	53.7 (45)	37.5 (40)	41.5 (30)
9	160 (50)	74 (50)	42.5 (50)
10	53 (40)	38.5 (45)	30.5 (45)
12	77.5 (40)	54.5 (45)	64 (50)

the human (~2.5 l), a booster dose of 100 µg oFSH may result in a circulating level of ~40 ng/ml. Since we followed a fixed injection schedule and antibody titration was done retrospectively, we learnt only at the end of the study that the boosters should not have been given on the days prescribed. We now believe that for the response to be good the boosters should be given only once in 100–120 days.

Though only 26–45% of the 'free' circulating antibodies generated to oFSH cross-reacted with hFSH, all volunteers exhibited such reactivity and the binding affinity was uniformly high ($0.65\times10^9 \pm 0.5\times10^9$ M^{-1}). The capacity of the antibody to bind endogenous FSH (hFSH) was high between days 30 and 80 of immunization and this concurs with the observation that no 'free FSH' was detectable between those days. Even in the earlier study in the monkey, no circulating 'free FSH' was detectable at time points when 'free' antibody was present (Aravindan *et al.*, 1993). In the receptor binding inhibition assay, used to determine bioneutralizing activity of the antibody, it was observed that a 50 µl aliquot of serum from an individual volunteer had maximal inhibitory activity in the range 73–95%, and this correlated with the high antibody titre existing at that time as determined by conventional binding procedures. Since our primary interest was to monitor the titre of the bioneutralizing type of antibodies, it may be desirable to consider % inhibition of binding to the receptor as a true measure of antibody titre. Considering that the concentrations of LH and TSH, as well as of testosterone, thyroxine and

triiodothyronine, remained unaltered following immunization, it can be concluded that the antibody produced is specific to FSH and does not react with the other pituitary glycoprotein hormones.

Immunization of men with oFSH using the dose schedule reported here did not result in any adverse physiological effects, since haematology, serum biochemistry and hormonal profiles remained within normal ranges and were unaltered. No evidence for production of autoantibodies to non-specific tissues was obtained. This essentially corroborates the results of the earlier studies in monkeys (Srinath *et al.*, 1983; Sehgal *et al.*, 1991), which indicated that, following immunization with FSH, occurrence of either immune complexes in the circulation or the presence of precipitated immune complexes in a variety of tissues was not apparent using immunofluorescence techniques.

With a view to obtaining an assessment of the ability of the antibody generated to block endogenous FSH action *in vivo*, sperm counts and quality and the seminal plasma transferrin concentration were determined. In the earlier monkey study it had been observed that sperm counts were significantly reduced only after 120 days of immunization, acute oligospermia and infertility arising by 200 days after immunization (Moudgal, 1981; Moudgal *et al.*, 1992). This suggests that the desired effect can been seen after continuous FSH deprivation for 3–4 sperm cycles. Considering that in the human one sperm cycle is ~60–65 days, we believe that, in the present study, endogenous FSH was neutralized for too short a time (~50–80 days) for us to observe a significant reduction in sperm production. The reduction in sperm counts that was observed in the immunized volunteers (33–64%) is therefore a first positive indication of what may occur (acute oligospermia) if deprivation of endogenous FSH support is maintained for a continuous period of >200 days.

Although Nieschlag (1985) also found that immunization of monkeys with oFSH led to severe impairment of spermatogenesis, he recommended abandoning this approach for contraception as the animals exhibited resurgence in spermatogenesis to some extent following repeat boosting over a 4.5 year period and azoospermia was not achieved. However, no fertility testing was undertaken by this group. While the partial resurgence in spermatogenesis observed could be due to repeat excessive boosting with oFSH in close succession, our studies (Moudgal *et al.*, 1992) as well as those of others (Raj *et al.*, 1991; Srivatsava and Das, 1992) in monkeys have clearly shown that oligospermia obtained as a consequence of FSH deprivation is compatible with establishment of infertility. A variety of parameters have been used to assess the quality of spermatozoa voided by monkeys immunized with oFSH. Besides routine indices such as sperm numbers, viability, motility, gel penetrability and sperm acrosin and hyaluronidase activity, which all showed significant reduction following immunization with oFSH for >6 months (Moudgal *et al.*, 1992; Moudgal and Aravindan, 1993), the spermatozoa of immunized monkeys were also incapable of binding to either homologous monkey oocytes (Raj *et al.*, 1991) or to zona-denuded hamster oocytes (Sharma and Das, 1992). Spermatozoa from immunized monkeys were also analysed for compaction of chromatin structure using flow cytometry. Two parameters were employed to determine this: (i) acridine orange binding to DNA following acid denaturation and (ii) ethidium bromide binding to DNA following treatment with dithiothreitol. The results clearly indicated that FSH deprivation had a marked effect on sperm chromatin compaction (Aravindan *et al.*, 1991). Evenson and colleagues (Ballachey *et al.*, 1988; Evenson, 1989) have demonstrated quantifiable differences in the quality of spermatozoa voided by proven fertile as compared to infertile human males as well as by control as compared to chemically induced infertile bulls. We therefore used the chromatin structure assay procedure as described by Evenson (1989) to determine whether FSH immunization affected the quality of spermatozoa voided by immunized human volunteers. The results showed a distinct pattern of change in decondensation of sperm chromatin structure, very similar to that exhibited by monkeys immunized with oFSH as well as by a category of infertile men (as reported by Evenson, 1989). Interestingly, the change in pattern was correlated with the antibody titres persisting at different periods of sperm sampling and analysis (H.Krishnamurthy, K.M. Prasanna Kumar and N.R.Moudgal, unpublished observation).

Based on studies on transferrin concentrations in seminal fluid from normal and vasectomized men, it has been suggested that >80% of seminal transferrin comes from the testis (Orlando *et al.*, 1985). Sertoli cells cultured in the presence of FSH stimulation are known to produce transferrin (Skinner and Griswald, 1992). Hence, the reduction in seminal transferrin observed in the FSH-immunized volunteers achieves significance, as it can be used as a parameter to assess Sertoli cell function in an FSH-deprived state. In support of this, the seminal plasma of long-term (>5 years) oFSH-immunized bonnet monkeys showed a 92% reduction (from 5.1 ± 0.8 to 0.38 ± 0.04 μg human transferrin equiv./ml; $P < 0.01$) in transferrin concentration compared with controls. Seminal transferrin is therefore likely to provide a reliable index of seminiferous tubular function since concentrations of this protein have been found to be low in patients exhibiting oligo- and azoospermia (Holmes *et al.*, 1982; Orlando *et al.*, 1985). It can therefore be inferred that in man Sertoli cell dysfunction can be achieved by immunization with oFSH.

The current study is a forerunner of a detailed clinical trial and shows that administration of oFSH, a heterologous hormonal protein, in a relatively mild adjuvant (Alugel) elicits a satisfactory immune response in adult human male volunteers. The antibodies generated are able to specifically bind to hFSH as well as bioneutralize the activity of endogenous FSH. The data also suggest that oFSH vaccine administered at the dose used here is relatively safe for human usage. It should be possible to increase the hFSH antibody titre, as well as prolong its bioefficacy, by giving a booster once in 100 days, instead of once in 20–30 days as used currently, and perhaps using recently discovered enhancers of adjuvant activity, such as sodium phthalyl derivative of lipopolysaccharide (SPLS) (Deshmukh and Talwar, 1995) or monophosphoryl lipid A (MPL) immunostimulant (Rudback *et al.*, 1995) already cleared for human use. Use of human instead of ovine FSH as an antigen in the human is feasible, but this would entail

administering hFSH conjugated to a heterologous protein or hapten. Intact FSH can conveniently be substituted by its β subunit, and oFSHβ in particular has a high degree of homology with hFSHβ; antibodies raised against it in monkeys have been shown to cross-react with both human and monkey FSH (Raj *et al.*, 1982; H.Krishnamurthy, S.Surekha, H.N.Krishnamurthy, G.S.Murthy and N.R.Moudgal, unpublished observation). When we started this study recombinant FSH was not available; such preparations for the human (Keene *et al.*, 1989) as well as ovine species (Samaddar *et al.*, 1996) now appear to be easy to procure, making it possible in the future to test such material in the human.

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