Human Reproduction vol.12 no.1 pp.59–63, 1997

Relationship between serum follicle stimulating hormone in the male and standard sperm parameters, and the results of intracytoplasmic sperm injection

V.Novero[3,4], M.Camus[1], H.Tournaye[1], J.Smitz[1], G.Verheyen[1], H.Joris[1], M.-P.Derde[2], A.C.Van Steirteghem[1] and P.Devroey[5]

[1]Centre for Reproductive Medicine and [2]Biomedical Statistics Department, University Hospital, Dutch-speaking Brussels Free University (Vrije Universiteit Brussel), Laarbeeklaan 101, 1090 Brussels, Belgium and [3]Department of Obstetrics and Gynecology, University of the Philippines, Philippine General Hospital, Taft Avenue, Metro Manila, Philippines

[4]Present address: Centre for Reproductive Medicine, University Hospital, Dutch-speaking Brussels Free University (Vrije Universiteit Brussel), Laarbeeklaan 101, 1090 Brussels, Belgium

[5]To whom correspondence should be addressed

Serum follicle stimulating hormone (FSH) is routinely measured when evaluating the infertile male for intracytoplasmic sperm injection (ICSI). However, among the sperm parameters, only its relationship with sperm concentration is well documented. Few investigations concern the relationship between FSH and sperm motility and morphology, and the results of ICSI. A retrospective study of 316 couples who underwent ICSI was carried out to determine the relationships between serum FSH concentrations in the male and (i) standard sperm parameters (concentration, motility and morphology) and (ii) fertilization, cleavage, pregnancy and implantation rates after ICSI. There was an inverse correlation with sperm concentration and total motility but no relationship was found with progressive motility and sperm morphology. Neither was any relationship found between serum FSH and fertilization, cleavage, pregnancy and implantation rates, and the results of ICSI. These findings suggest the need to review the routine measurement of serum FSH in the infertile male when ICSI is the planned treatment procedure.

Key words: follicle stimulating hormone/intracytoplasmic sperm injection/male infertility/microinjection

Introduction

Because of its physiological role in spermatogenesis, serum follicle stimulating hormone (FSH) has for a long time been accepted as a standard laboratory test in the evaluation of the infertile male. Upon completion of the patient's history, a physical examination and the detection of an abnormal semen analysis, the measurement of FSH is usually the next step in evaluating male factor infertility (Rodriguez-Rigau, 1983; Herlichy *et al.*, 1987). Although accepted as part of any routine diagnostic work-up, it is commonly used as a predictor of achieving success in in-vivo fertilization (Silber, 1989).

Most of the studies performed on the predictive role of FSH with regard to the success of fertility treatment have been based on sperm concentration. There are, so far, no investigations on its relationship to sperm motility, morphology and especially the various outcome measures of standard in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), otherwise known as microinjection.

The discovery of microinjection revolutionized the treatment of male infertility. Males previously thought to be absolutely sterile can now reasonably expect some degree of success in terms of fertilization and implantation (Van Steirteghem *et al.*, 1993a,b). Successful pregnancies by microinjection have been achieved utilizing only a few spermatozoa obtained from ejaculated semen from the epididymis or directly from testicular tissue (Devroey *et al.*, 1994; Silber *et al.*, 1994, 1995; Nagy *et al.*, 1995a).

The aim of this study was to analyse the relationship between serum FSH in the male and sperm concentration, motility and morphology. Subsequently its relationship to the results of microinjection (fertilization, cleavage, pregnancy and implantation rates) were analysed in 316 couples who underwent ICSI between January and September 1994.

Materials and methods

Patients

A total of 1058 couples who underwent ICSI between January and September 1994 were screened for the study; some 316 couples were included in the final study. Only those couples in which the husband had had a serum FSH determination at our laboratory were selected. Couples who utilized frozen or donor spermatozoa were excluded from the study.

The patients' inclusion criteria for microinjection were either failed or very low fertilization rates in their previous standard IVF cycle(s) or extremely poor semen parameters (<500 000 motile spermatozoa with normal morphology in the whole ejaculate).

Ovarian stimulation

Ovarian stimulation was carried out by a desensitizing protocol using gonadotrophin-releasing hormone agonist buserelin (Suprefact; Hoechst, Brussels, Belgium) in combination with human menopausal gonadotrophins (HMG; Humegon; Organon, Oss, The Netherlands; or Pergonal; Serono, Brussels, Belgium) and human chorionic gonadotrophins (HCG; Pregnyl, Organon; Profasi, Serono). Intravaginally administered progesterone (Utrogestan; Piette, Brussels, Belgium) was used for luteal phase supplementation. The details of this stimulation protocol have been described previously (Smitz *et al.*, 1992).

Semen evaluation and preparation

Sperm concentration and motility were evaluated according to the recommendations of the World Health Organization (WHO, 1992).

Morphology was evaluated after Shorr staining by strict Kruger criteria (Kruger *et al.*, 1986).

The treatment procedure of the semen has been described previously (Liu *et al.*, 1994). Semen was washed in Earle's medium by centrifuging for 5 min at 1800 *g* after a 30 min liquefaction period. The pellet was put on a two-layer Percoll gradient (95–47.5%) and was then centrifuged at 300 *g* for 20 min. The 95% Percoll fraction was washed again with Earle's medium for 5 min at 1800 *g* and the pellet recentrifuged in Earle's medium just prior to microinjection.

Oocyte preparation

Cumulus–corona–oocyte complexes (CCOC) were retrieved by vaginal ultrasound-guided puncture performed 36 h after HCG administration. The cells of the cumulus and corona radiata were removed by incubation of the CCOC for <1 min in HEPES-buffered Earle's medium containing 80 IU/ml hyaluronidase (Type VIII; specific activity 320 IU/mg; Sigma Chemical Co., St Louis, MO, USA) and by aspiration of the cumulus complexes in and out of a hand-drawn glass pipette. The denuded oocytes were rinsed several times, first in HEPES-buffered Earle's medium and then in B2 medium (BioMérieux, Montalieu Vercieu, France). The nuclear maturity of the oocytes was judged under an inverted microscope at ×200 magnification. Until the moment of injection, the oocytes were kept in 25 ml microdrops of B2 medium covered by lightweight paraffin oil (British Drug House, Pasteur, Brussels, Belgium) in an incubator at 37°C in an atmosphere of 5% CO_2, 5% O_2 and 90% N_2. ICSI was carried out only on metaphase II oocytes (Van Steirteghem *et al.*, 1993a,b).

ICSI procedure

Details of the microtool preparation and microinjection procedures have been described previously (Van Steirteghem *et al.*, 1993a,b).

A single, living, immobilized spermatozoon was aspirated tail first into the injection pipette. For the injection, the oocyte was fixed on the holding pipette so that the polar body was situated at 12 or 6 o'clock. Meanwhile the injection pipette was pushed through the zona pellucida at the 3 o'clock position and into the cytoplasm, where the spermatozoon was delivered together with ~1 pl medium.

After injection, the oocytes were washed and stored in 25 ml microdrops of B2 medium in a Petri dish and stored in an incubator containing 5% CO_2, 5% O_2 and 90% N_2.

Assessment of fertilization, embryo cleavage and pregnancy

At 16–18 h after microinjection, oocytes were inspected for survival and fertilization (Nagy *et al.*, 1994). The number and aspect of polar bodies and pronuclei were recorded. The criteria for normal fertilization were the presence of two separate or fragmented polar bodies, together with two clearly visible pronuclei. Embryo cleavage and quality were evaluated 40–44 h after microinjection. According to the relative proportion of anucleate fragments present in the zona pellucida, they were assigned to one of the four categories: (i) excellent, when no anucleate fragment was present; (ii) good, when <20% of the embryo was fragmented; (iii) fair, relative fragmentation 20–50%; and (iv) poor, >50% fragmentation. Embryos with <50% fragmentation were eligible for transfer. As a rule, a maximum of three embryos were replaced. In exceptional circumstances, when the woman's age was ⩾40 years, a maximum of five embryos were replaced. To avoid multiple pregnancies, especially triplet pregnancies, only two embryos were transferred in some selected cases (Staessen *et al.*, 1993).

Pregnancy was detected when serum HCG concentrations were rising on at least two separate occasions 10 days after embryo transfer. Clinical ongoing pregnancy was determined by detecting a gestational sac by transvaginal ultrasonography at 7 weeks of pregnancy.

Determination of serum FSH

Serum FSH concentrations in the male were measured using the standardized immunoradiometric kit [^{125}I]hFSH Coatria (BioMérieux, Marcy l'Etoile, France) in the laboratory of the Dutch-speaking Brussels Free University (Brussels, Belgium). The reference range established for the immunoassay kit at the time of the study was 1.2–11.0 mIU/ml. A single determination of FSH was utilized for each patient.

Data set and statistics

For descriptive purposes and to present the results more clearly, five categories of FSH concentration were created. These were: group A (FSH <1.2 mIU/ml), which is below the minimum level of normal range for the immunoassay kit used in the laboratory; group B (FSH = 1.2–11.0 mIU/ml), representing the normal range; group C (FSH = 11.1–15.0 mIU/ml); group D (FSH = 15.1–25.0 mIU/ml); and group E (FSH level >25.0 mIU/ml). The last three categories are the arbitrarily graded groups of elevated FSH concentration. Based on the above scheme, three couples were placed in group A, 241 in group B, 28 in group C, 29 in group D and 15 in group E. For statistical testing, the actual serum FSH concentrations were considered.

Statistical tests were performed two-sided at the 5% level of significance. Calculations were carried out using the SPSS statistical package on an Inwork personal computer.

The relationships between serum FSH and sperm concentration, total motility, progressive motility and sperm morphology were quantified globally using the Spearman rank correlation coefficient. The same approach was used to quantify the relationship between serum FSH and outcome measures such as fertilization, cleavage and implantation rates. The relationships between serum FSH and (i) the number of cycles with an embryo transfer and (ii) pregnancy rate were investigated by comparing the median FSH concentrations in cycles with and without an embryo transfer and in patients becoming and not becoming pregnant respectively, using the Mann–Whitney *U*-test.

Results

Serum FSH and sperm parameters

The median values of the different sperm parameters according to serum FSH concentration are summarized in Table I. The serum concentration of FSH in the male was correlated inversely with median sperm concentration ($P < 0.001$). Beginning at 32×10^6/ml for group A, the sperm concentration decreased as the FSH concentration increased, until it reached 0.1×10^6/ml at the highest FSH concentration in group E.

Serum FSH concentrations were also correlated inversely with total sperm motility ($P = 0.036$). In the presence of high FSH concentrations >25 mIU/ml, the median total motility decreased to 7%. Progressive sperm motility and sperm morphology were not significantly correlated with serum FSH. For morphology in particular, the percentage normal spermatozoa using median values was nearly constant at 5–6% in all five FSH groups.

Serum FSH and outcome measures of ICSI

Oocyte number, intactness after ICSI, fertilization rate and cleavage rate in the various FSH groups are summarized in

Table I. Median values and ranges of sperm parameters according to various concentrations of serum follicle stimulating hormone (FSH)

	FSH concentration (mIU/ml)					*P* value (Spearman rank) *r*
	A <1.2	B 1.2–11.0	C 11.1–15.0	D 15.1–25.0	E >25.0	
Sperm concentration ($\times 10^6$/ml)	32.2 (2–47)	9.9 (0–280)	6.0 (0–26)	0.8 (0–43)	0.1 (0–7.6)	<0.001 –0.399
Motility (%)	35 (17–37)	40 (0–100)	40 (0–76)	44 (0–100)	7 (0–80)	0.036 –0.128
Progressive motility (%)	15 (7–22)	30 (0–86)	28 (0–66)	32 (0–72)	7 (0–80)	NS –0.095
Sperm morphology (%)	6 (2–11)	6 (0–97)	6 (0–25)	5 (0–23)	6 (2–20)	NS –0.081

Values in parentheses are ranges. NS = not significant.

Table II. Mean values of fertilization and cleavage rates per cycle according to various concentrations of serum follicle stimulating hormone (FSH)

	FSH concentration (mIU/ml)					*P* value (Spearman rank) *r*
	A <1.2	B 1.2–11.0	C 11.1–15.0	D 15.1–25.0	E >25.0	
No. of cycles	3	241	28	29	15	
No. of injected oocytes	12.0	11.4	11.6	9.7	12.9	
No. of intact oocytes	12.0	10.4	10.2	8.7	11.8	–0.061 (NS)
No. of two-pronuclear oocytes	10.0	7.85	7.7	5.8	8.0	–0.075 (NS)
% of two-pronuclear oocytes	81.0	75.6	70.6	66.0	68.5	–0.067 (NS)
No. of transferable embryos	6.7	5.9	5.7	4.2	4.9	–0.032 (NS)
Cleavage of two-pronuclear oocytes (%)	66.1	75.5	72.1	78.2	57.2	0.100 (NS)

NS = not significant.

Table III. Pregnancy rates according to various concentrations of serum follicle stimulating hormone (FSH)

	FSH concentration (mIU/ml)					*P* value (Mann–Whitney *U*-test)
	A <1.2	B 1.2–11.0	C 11.1–15.0	D 15.1–25.0	E >25.0	
No. of cycles	3	241	28	29	15	
No. of transfers	3	234	27	28	10	
Transfer rate (%)	100	97	96	97	67	0.004
No. of positive HCG tests	3	101	13	12	6	
% per ovum retrieval	100.0	41.9	46.4	41.4	40.0	NS
% per transfer	100.0	43.2	48.1	42.9	60.0	NS
No. of ongoing pregnancies or deliveries	3	86	12	11	6	
% per oocyte retrieval	100.0	35.7	42.9	37.9	40.0	NS
% per transfer	100.0	36.8	44.4	39.3	60.0	NS

HCG = human chorionic gonadotrophin; NS = not significant.

Table II. The two-pronuclear fertilization rate varied from 81.0 (FSH <1.2 mIU/ml) to 66.0% (FSH 15.1–25.0 mIU/ml). No statistical correlation was found between serum FSH concentration and the two-pronuclear fertilization rate. The mean cleavage rate of the normally fertilized oocytes varied from 57.2 to 78.2% in the different serum FSH groups, and these were not found to be significantly different.

The initial pregnancy and combined ongoing pregnancy/delivery rates in the five different FSH groups are presented in Table III. The rates were similar either per oocyte retrieval or per embryo transfer. They were found to be not significantly different by the Mann–Whitney *U*-test. However, the embryo transfer rate was found to be inversely associated with the serum concentration of FSH, where values ranged from 100% at FSH <1.2 mIU/ml to 67% at FSH >25.0 mIU/ml.

The results concerning implantation are shown in Table IV. The implantation rate, defined as the number of gestational sacs per transferred embryo, was found to be not significantly different when related to the different serum FSH concentrations. This was calculated using the Spearman rank correlation coefficient.

Discussion

It is generally accepted that the chance of achieving a spontaneous pregnancy is increased when FSH concentrations in the

Table IV. Implantation according to various concentrations of serum follicle stimulating hormone (FSH)

	FSH concentration (mIU/ml)					*P* value (Spearman rank) *r*
	A <1.2	B 1.2–11.0	C 11.1–15.0	D 15.1–25.0	E >25.0	
No. of cycles	3	241	28	29	15	
No. of embryo transfers	3	234	27	28	10	
Total no. of embryos transferred	7	609	69	65	26	
Mean no. of transferred embryos/transfer	2.33	2.53	2.46	2.24	1.73	–0.040 (NS)
Total no. of gestational sacs	3	125	14	14	9	
Implantation rate (sacs/embryos transferred)	44.4	20.9	18.8	22.6	36.7	0.073 (NS)

NS = not significant.

male are normal. Conversely, an elevated FSH concentration in the male is equated with infertility and sterility. Based on these principles, serum FSH is routinely measured to aid treatment planning for the infertile male.

In general these principles are based on ample evidence that an inverse relationship exists between sperm concentration and FSH concentration in the male (Rosen and Weintraub, 1971; Kjessler and Wide, 1973; Mauss and Börsch, 1973; Hunter *et al.*, 1974; Christiansen, 1975; Aafjes *et al.*, 1977; Wu *et al.*, 1981), although was not confirmed by others (de Kretser *et al.*, 1972; Franchimont *et al.*, 1972; Leonard *et al.*, 1972). Our results positively confirm this relationship. Moreover, our data demonstrate an inverse relationship between serum FSH concentration and percentage total sperm motility. However, there is no correlation whatsoever between serum FSH concentration and progressive sperm motility or sperm morphology.

Although traditional IVF was first used successfully in male infertility some 12 years ago (Mahadevan *et al.*, 1983), success in standard IVF is still dependent on a sufficient and minimum number of sperm cells that can be retrieved from ejaculated spermatozoa after swim-up or Percoll treatment (Duncan *et al.*, 1993). For this reason, the selection of patients for IVF or ICSI may be based merely on this critical number of retrievable spermatozoa without a measure of serum FSH.

As demonstrated in our study, sperm concentration becomes extremely low when FSH concentrations are elevated significantly. Because sperm numbers are related to the success of fertilization *in vivo* and in standard IVF, and serum FSH is related to sperm numbers, FSH may have a predictive value for the success of IVF, i.e. when the FSH concentration is elevated, the probability of fertilization is decreased in natural procreation and standard IVF. However, as far as we know, no published data are available comparing the FSH concentration in the male with the results of standard IVF.

Ever since ICSI became available in 1992, males with extremely impaired semen samples, including those with cryptozoospermia, asthenozoospermia and/or teratozoospermia, have been treated successfully (Nagy *et al.*, 1995a,b). In our study, comparable success rates were achieved regardless of the serum FSH concentration. As shown previously, fertilization, cleavage, pregnancy and implantation rates were totally independent of serum FSH (and sperm) concentration when ICSI was performed. Therefore, FSH in the male seems to be of no value in predicting success in ICSI.

The absence of a relationship between serum FSH and the other sperm parameters (progressive motility and sperm morphology), and between serum FSH and the results of ICSI, is consistent with a recent study in which various sperm impairments were found to have no influence on the outcome of ICSI (Nagy *et al.*, 1995a). Therefore microinjection is not similar to standard IVF, whose results are influenced by sperm count and progressive sperm motility (Duncan *et al.*, 1993) and sperm morphology (Kruger *et al.*, 1988).

Because of this absence of a relationship between FSH concentration in the male and the outcome measures of ICSI, our study challenges the value of measuring FSH concentration in the infertile male utilizing ICSI. If the sperm concentration after swim up is $>1\times10^6$/ml, then the patient may be eligible for regular IVF. But in those patients with an extremely low sperm concentration predicting an IVF failure, ICSI would be the logical alternative. In such cases, the determination of serum FSH would not provide any added clinical importance regarding the success of ICSI treatment. Those data demonstrate the relative unimportance of FSH measurements in the infertile male (Martin-du-Pan and Bischof, 1995).

This study clearly demonstrates that when using ICSI, knowing the serum FSH concentration in the male has no value in predicting the results. However, in cases of azoospermia, the measurement of FSH may be important in distinguishing obstructive from non-obstructive azoospermia. Normal serum FSH and azoospermia are diagnostic of excretory duct obstruction (Wu *et al.*, 1981). On the other hand, the FSH concentration is elevated in the non-obstructive type (Wu *et al.*, 1981; Micic *et al.*, 1983). From a counselling viewpoint, though, determining the serum FSH concentration is important to differentiate obstructive from non-obstructive azoospermia. This differentiation has some importance in determining the approach to the future treatment of male infertility but not to the success of ICSI.

In summary, our study has confirmed the inverse relationship between serum FSH concentration and sperm concentration. Furthermore, it has shown that no relationship exists between serum FSH concentration and progressive sperm motility, sperm morphology and the results (fertilization, cleavage, implantation and pregnancy rates) of ICSI. The need to measure serum FSH concentration in the male has to be reviewed in patients being prepared for ICSI because FSH will be of no benefit in predicting the success or failure of treatment. It may,

however, be useful in distinguishing obstructive from non-obstructive azoospermia.

Acknowledgements

We are indebted to all our colleagues at the Centre for Reproductive Medicine, Prof. Michael Whitburn of the Language Education Centre and Mrs Sofie De Moor for typing the manuscript. This work was supported by grants from the Belgian Fund for Medical Research.

References

Aafjes, J.H., Van Der Vijver, J.C.M., Docter, R. *et al.* (1977) Serum gonadotrophins, testosterone, and spermatogenesis in subfertile men. *Acta Endocrinol.*, **86,** 651–658.

Christiansen, P. (1975) Studies on the relationship between spermatogenesis and urinary levels of follicle stimulating hormone and luteinizing hormone in oligospermic men. *Acta Endocrinol.*, **78,** 192–208.

de Kretser, D.M., Burger, H.G., Fortune, D. *et al.* (1972) Hormonal, histological and chromosomal studies in adult males with testicular disorders. *J. Clin. Endocrinol. Metab.*, **35,** 392–401.

Devroey, P., Liu, Y., Nagy, Z. *et al.* (1994) Normal fertilization of human oocytes after testicular sperm extraction and intracytoplasmic sperm injection. *Fertil. Steril*., **62,** 639–641.

Duncan, W.W., Glew, M.J., Wang, X.J. *et al.* (1993) Prediction of in-vitro fertilization rates from semen variables. *Fertil. Steril*., **59,** 1233–1238.

Franchimont, P., Millet, D., Vendrely, E. *et al.* (1972) Relationship between spermatogenesis and serum gonadotropin levels in azoospermia and oligospermia. *J. Clin. Endocrinol. Metab.*, **34,** 1003–1008.

Herlichy, R., Lee, R.D. and Lipshultz, L.I. (1987) Evaluation of the infertile male. In Pryor, J.P. and Lipshultz, L.I. (eds), *Andrology.* Butterworth & Co. Ltd, London, UK, pp. 242–260.

Hunter, W.M., Edmond, P., Watson, G.S. *et al.* (1974) Plasma LH and FSH levels in subfertile men. *J. Clin. Endocrinol. Metab.*, **39,** 740–749.

Kjessler, B. and Wide, L. (1973) Follicle stimulating hormone (FSH) and interstitial cell stimulating hormone (ICSH) in relation to gametic output in 643 males. *Acta Endocrinol*., **72,** 243–256.

Kruger, T.F., Menkveld, R., Stander, F.S.H. *et al.* (1986) Sperm morphologic features as a prognostic factor in in-vitro fertilization. *Fertil. Steril.*, **46,** 1118–1123.

Kruger, T.F., Acosta, A.A., Simmons, K.F. *et al.* (1988) Predictive value of abnormal sperm in in-vitro fertilization. *Fertil. Steril*., **49,** 112–117.

Leonard, J.M., Leach, R.B., Couture, M. *et al.* (1972) Plasma and urinary follicle-stimulating hormone levels in oligospermia. *J. Clin. Endocrinol*., **34,** 209–214.

Liu, J., Nagy, Z.P., Joris, H. *et al.* (1994) Intracytoplasmic sperm injection does not require special treatment of the spermatozoa. *Hum. Reprod.*, **9,** 1127–1130.

Mahadevan, M.M., Trounson, A.O. and Leeton, J.F. (1983) The relationship of tubal blockage, infertility of unknown cause, suspected male infertility, and endometriosis to success of in vitro fertilization and embryo transfer. *Fertil. Steril.*, **40,** 755–762.

Martin-du-Pan, R.C. and Bischof, P. (1995) Increased follicle stimulating hormone in infertile men: is increased plasma FSH always due to damaged epithelium? *Hum. Reprod.*, **10,** 1940–1945.

Mauss, J. and Börsch, G. (1973) Immunochemical assay of follicle-stimulating hormone in the unconcentrated urine of sub or infertile males. *Acta Endocrinol.*, **74,** 631–641.

Micic, S., Ilic, V., Micic, M. *et al.* (1983) Endocrine profile of 45 patients with Sertoli cell only syndrome. *Andrologia*, **15,** 228–232.

Nagy, Z.P., Liu, J., Joris, H. *et al.* (1994) Time course of oocyte activation, pronucleus formation and cleavage in human oocytes fertilized by intracytoplasmic sperm injection. *Hum. Reprod.*, **9,** 1743–1748.

Nagy, Z.P., Liu, J., Janssenswillen, C. *et al.* (1995a) Using ejaculated, fresh, and frozen–thawed epididymal and testicular spermatozoa gives rise to comparable results after intracytoplasmic sperm injection. *Fertil. Steril.*, **63,** 808–815.

Nagy, Z.P., Liu, J., Joris, H. *et al.* (1995b) The result of intracytoplasmic sperm injection is not related to any of the basic three sperm parameters. *Hum. Reprod.*, **10,** 1123–1129.

Rodriguez-Rigau, L.J. (1983) Endocrine evaluation of the infertile male. In Negro-Vilar, A. (ed.), *Male Reproduction and Fertility.* Raven Press, New York, NY, USA, pp. 257–263.

Rosen, S.W. and Weintraub, B.D. (1971) Monotropic increase of serum FSH correlates with low sperm count in young men with idiopathic oligospermia and aspermia. *J. Clin. Endocrinol. Metab.*, **32,** 410–427.

Silber, S.J. (1989) The relationship of abnormal semen parameters to male fertility. *Hum. Reprod.*, **4,** 947–953.

Silber, S.J., Nagy, Z.P., Liu, J. *et al.* (1994) Conventional IVF versus ICSI for patients requiring microsurgical sperm aspiration (MESA). *Hum. Reprod.*, **9,** 1705–1709.

Silber, S.J., Van Steirteghem, A.C., Liu, J. *et al.* (1995) High fertilization and pregnancy rates from intracytoplasmic sperm injection using spermatozoa obtained from testicle biopsy. *Hum. Reprod.*, **10,** 148–154.

Smitz, J., Devroey, P., Faguer, B. *et al.* (1992) A prospective randomized comparison of intramuscular or intravaginal natural progesterone as a luteal phase and early pregnancy supplement. *Hum. Reprod.*, **7,** 168–175.

Staessen, C., Janssenswillen, C., Van den Abbeel, E. *et al.* (1993) Avoidance of triplet pregnancies by elective transfer of two good-quality embryos. *Hum. Reprod.*, **8,** 1650–1653.

Van Steirteghem, A.C., Liu, J., Joris, H. *et al.* (1993a) Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report of a second series of 300 consecutive cycles. *Hum. Reprod.*, **8,** 1055–1060.

Van Steirteghem, A.C., Nagy, Z.P., Joris, H. *et al.* (1993b) High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum. Reprod.*, **8,** 1061–1066.

World Health Organization (1992) *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction.* 3rd edn, Cambridge University Press, Cambridge, UK.

Wu, F.C.W., Edmond, P., Raab, G. *et al.* (1981) Endocrine assessment of the subfertile male. *Clin. Endocrinol.*, **14,** 493–507.

Received on September 5, 1995; accepted on October 23, 1996