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Accuracy of Sperm Characteristics in Predicting the in Vitro Fertilizing Capacity of Semen

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Based on the results of in vitro fertilization (IVF) in 56 couples, the power was assessed of traditional sperm characteristics of native semen to discriminate between in vitro fertile and in vitro infertile semen. The number per ejaculate of spermatozoa with regular oval heads was the best discriminant, followed by the concentration of progressively motile spermatozoa. This contrasts with the in vivo fertilizing capacity, which depends mostly on the proportion and concentration of spermatozoa with rapid linear progression. The lower limit of sperm characteristics was assessed as the fifth percentile of in vitro fertile semen and was compared with the lower limit of semen of fertile men and of subfertile men who achieved spontaneous or treatment-related conception in vivo. It appeared that the semen quality needed for in vitro fertilization is inferior to that of fertile men but not remarkably different from that of subfertile men who achieved spontaneous conception during 1-year follow-up after consultation. If conventional methods for semen preparation are used, there seems to be no major advantage in favor of IVF for the treatment of male infertility due to sperm deficiency. An increased success rate may, however, be attained, thanks to improved techniques of semen collection, semen preparation, and oocyte insemination.

KEY WORDS: male infertility; in vitro fertilization (IVF); receiver operating characteristic (ROC) curves; semen quality.

INTRODUCTION

In vitro fertilization (IVF) has been advocated as a possible treatment for male infertility (1,2). Although the ovum-fertilization rate with subnormal semen was lower than with normal semen, the success rate in terms of pregnancies was satisfactory. IVF is applicable only in subfertile men who have spermatozoa present in their ejaculate, although these are of abnormal quality and/or in low numbers. The success rate of IVF should be compared with the conception rate in untreated couples (3–5) or couples undergoing other treatments such as intrauterine or intraperitoneal insemination with husband semen (6–8).

In order to study the accuracy of sperm characteristics in predicting the in vitro fertilizing capacity of semen and to assess the role of IVF in the treatment of male infertility, we have analyzed the characteristics of semen which did or did not fertilize in vitro and calculated the lower limits of in vitro fertile semen. The latter values were compared to values reported for in vivo fertile semen (9).

MATERIALS AND METHODS

The characteristics of spermatozoa were assessed in semen of 56 couples who consulted for infertility due to either male or female causes and who were referred for in vitro fertilization.

The IVF procedures were performed as described by Edwards (10). Ovarian stimulation was achieved by the administration of clomiphene citrate and human menopausal gonadotropin (Humegon, Organon, The Netherlands). The response to the stimulation was monitored by means of pelvic ultrasonography and measurements of serum estradiol, progesterone, and luteinizing hormone (LH) using radioimmunoassays. Ovulation was induced by the administration of 10,000 IU of human chori-

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onic gonadatropin (Pregnyl, Organon, The Netherlands) at an appropriate time, and oocyte retrieval was performed 34 to 36 hr later. Oocytes were aspirated through laparoscopy under general anesthesia and graded for maturity in accordance with the criteria described by Veeck *et al.* (11).

Semen collection and preparation were performed as described by Cohen *et al.* (2). In short, motile spermatozoa were recovered by means of repeated centrifugation and resuspension in Earle's medium supplemented with 10% decomplemented cord serum. Insemination was performed depending on oocyte maturity. Between 50,000 and 100,000 spermatozoa were used for insemination. Fertilization was defined by the presence of two or more pronuclei 16 to 20 hr after insemination.

In 43 of 56 cases (83%) fertilization of at least one oocyte was achieved. Eighty-seven percent of the fertilized ova which were not replaced cleaved. The pregnancy rate was 23% per pickup.

Analysis of the native semen included assessment of the ejaculate volume, counting of the sperm concentration with a hemocytometer, calculation of the total sperm count per ejaculate, and assessment of the characteristics of motility, viability, and morphology. Motility estimated using the conventional method in accordance with World Health Organization standards (14) was objectively assessed using a computer-assisted technique (AutoSperm, AMSATEN Corp., De Pinte, Belgium) (13).

Motility was classified into the following categories (14).

Grade a: Rapid linear progressive motility corresponding with a linear velocity $\geq$22 μm/sec.

Grade b: Sluggish linear or nonlinear progression corresponding with a linear velocity <22 μm/sec and a velocity $\geq$5 μm/sec.

Grade c: Nonprogressive motility corresponding with a velocity <5 μm/sec.

Grade d: Immotile spermatozoa.

Morphology was assessed on Papanicolaou-stained smears observed under negative phase-contrast illumination. World Health Organization standards were used to classify spermatozoa into morphologically normal or abnormal, including all elements of the head, midpiece, and tail (14).

Receiver operating characteristic (ROC) curves were used to assess the ability of each semen characteristic to discriminate between in vitro fertile and in vitro infertile semen (9,15,16). The curves are constructed from the cumulative frequency distribution of a particular semen characteristic in the two populations by plotting the proportion of subjects in the first group with values less than a given value x against the proportion in the second group with values less than x. If the distribution of the particular characteristic does not differ in both groups, the ROC curve will coincide with the diagonal. The greater the difference in the distribution of the characteristic in the two groups, the further

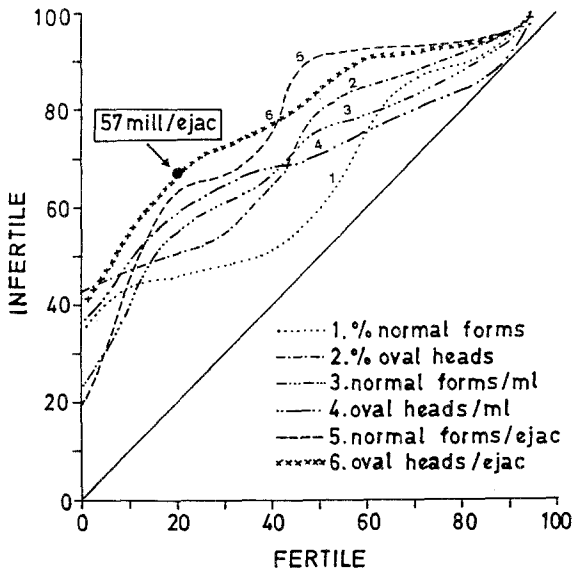

Fig. 1. Receiver operating characteristic (ROC) curves of sperm morphology characteristics of the in vitro fertile compared to the in vitro infertile population.

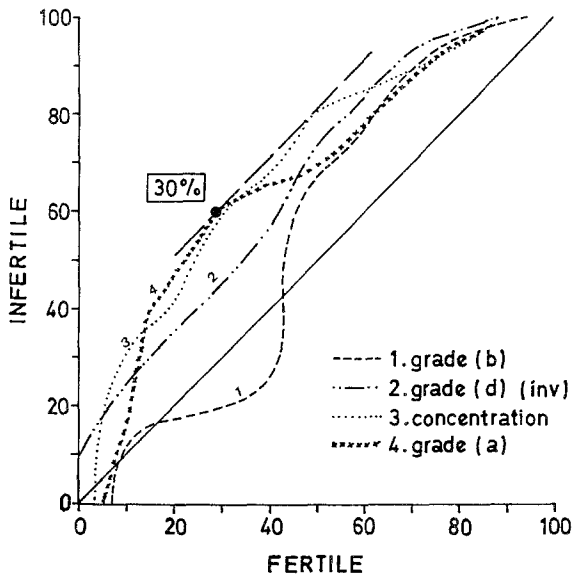

Fig. 2. ROC curves of semen characteristics of the in vitro fertile compared to the in vitro infertile population.

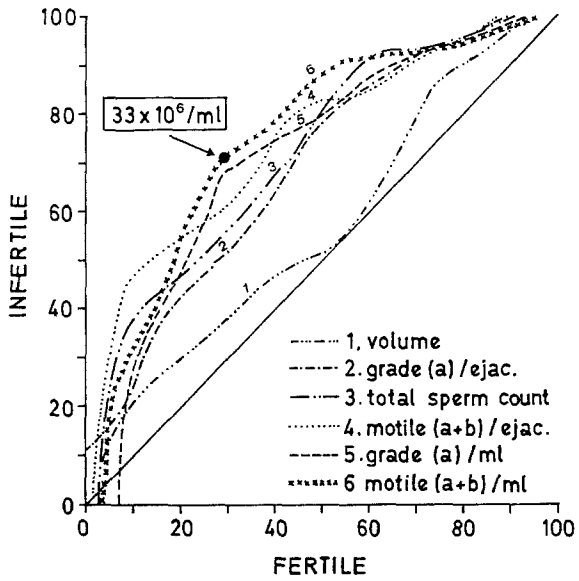

Fig. 3. ROC curves of semen characteristics of the in vitro fertile compared to the in vitro infertile population.

the curve will shift from the diagonal to the upper left corner. The distance from the diagonal to the observed ROC curve is a measure of the power of that characteristic to discriminate between the two groups. The measuring value located at the greatest distance from the diagonal is the criterion value which permits the best separation between the two groups. Using this criterion value, specificity and sensitivity as well as overall efficiency were calculated (17).

Based on the frequency distributions of the in vitro fertile population, the percentiles were calculated.

RESULTS

The receiver operating characteristic curves are shown in Figs. 1, 2, and 3, and the criterion values with the lowest error rate are shown in Table I. The best discriminant between in vitro fertile and in vitro infertile semen is the total number of spermatozoa with oval heads per ejaculate, followed by the concentration of progressively motile spermatozoa, grades a plus b added. The most sensitive test was the total sperm count, with only 8% false-negative results, whereas the percentage of spermatozoa with oval heads was the most specific test.

Based on the cumulative frequency distribution of sperm characteristics of the in vitro fertile group, percentiles were calculated (Table II). These were compared with the percentiles of semen of fertile men and of subfertile men who achieved pregnancy within 1 year after consultation for infertility (Tables III and IV) (9).

DISCUSSION

The results of this study must be interpreted with caution since the number of observations is small, which may interfere with the values for the lower

Table I. Criterion Values and Accuracy Parameters for Sperm Characteristics in Vitro

	Criterion value	Overall predictive value	Sensitivity	Specificity
Oval heads count (million/ejaculate)	57	73.5	67	80
Normal forms count (million/ejaculate)	39	72.5	63	82
Oval heads (%)	18	71.5	43	100
Grades a + b motile sperm concentration (million/ml)	33	71.0	71	71
Oval heads concentration (million/ml)	12	70.5	53	88
Grade a motile sperm concentration (million/ml)	23	69.0	68	70
Grades a + b motile sperm count (million/ejaculate)	54	68.5	47	90
Normal forms concentration (million/ml)	10	68.0	52	84
Normal forms (%)	5	67.0	34	100
Total sperm count (million/ejaculate)	300	66.0	92	40
Grade a motility (%)	30	66.0	60	72
Sperm concentration (million/ml)	93	64.5	80	49
Grade a motile sperm count (million/ejaculate)	32	64.0	78	50
Grade d motility (%)	53	61.5	76	47
Grade b motility (%)	19	59.5	87	32
Volume (ml)	4.2	56.5	87	26

Table II. Description of Sperm Characteristics of Men Whose Semen Was Fertile in Vitro (Median, Range, and Percentiles)

	Median	Range	5 P[a]	10 P[a]	90 P[a]	95 P[a]
Volume (ml)	3	1–8	1	2	6	7
Sperm concentration (million/ml)	92	4–251	20	31	200	221
Sperm count (million/ejaculate)	240	8–994	60	100	666	750
Grade a motility (%)	36	1–80	1	10	60	70
Grade a motile sperm concentration (million/ml)	32	0.04–168	1	3	78	95
Grade a motile sperm count (million/ejaculate)	97	0.08–790	5	10	385	531
Grades a + b motility (%)	53	21–86	22	30	76	77
Grades a + b motile sperm concentration (million/ml)	48	1–188	6	14	102	111
Grades a + b count (million/ejaculate)	143	2–833	18	54	492	532
Grade b motility (%)	15	2–35	3	6	28	30
Grade c motility (%)	6	0–17	0	0	10	13
Grade d motility (%)	41	14–73	19	22	57	60
Normal forms (%)	26	5–58	9	13	48	52
Normal forms concentration (million/ml)	28	0.2–138	2	5	53	138
Normal forms count (million/ejaculate)	89	0.4–378	7	15	196	231
Oval heads (%)	48	18–70	19	25	59	63
Oval heads concentration (million/ml)	36	6–108	7	10	85	102
Oval heads count (million/ejaculate)	102	17–630	20	36	293	352

[a] Fifth, tenth, ninetieth, and ninety-fifth percentiles.

limit of fertility, and since the absence of fertilization due to factors of the oocyte is not taken into account.

In contrast with the situation in vivo, where the percentage and concentration of grade a motile spermatozoa are the best discriminants (9), sperm morphology, and in particular head morphology, is the best discriminant in vitro. The second-best discriminant is the concentration of spermatozoa with progressive motility, including both grade a and grade b motility.

These findings are in agreement with results reported by others (18,19) and could be expected logically. Indeed, in vivo fertilization requires the spermatozoa first to migrate through the cervical mucus, which strongly depends on their rapid linear progression. In vitro, any progressive motility may suffice to penetrate the corona. The capacity of the sperm head to fuse with the ovum is expected to be more important. The latter should be better in spermatozoa with a morphologically normal head than in those with an abnormal head structure (20).

Comparison of the lower limit of normality (fifth percentile) of semen which is fertile in vivo versus in vitro reveals minor differences for sperm count or concentration. A lower degree of sperm motility is required for in vitro fertilization. If the results of in vitro fertilization are compared with those found in subfertile men who originally consulted for infertility but ultimately achieved conception within 12 months of the in vivo trial, there is no evident advantage in favor of the in vitro results. However,

Table III. Description of Sperm Characteristics of Semen of Men Who Were Fertile in Vivo (Median, Range, and Percentiles) (9)

	Median	Range	5 P[a]	10 P[a]	90 P[a]	95 P[a]
Sperm concentration (million/ml)	100	22–360	35	42	225	280
Sperm count (million/ejaculate)	280	66–1153	87	110	660	880
Grade a motility (%)	55	11–76	28	36	70	73
Grade a motile sperm concentration (million/ml)	56	10–208	15	21	120	150
Grades a + b motility (%)	67	28–92	48	52	80	83
Grades a + b motile sperm concentration (million/ml)	63	13–231	21	27	145	175
Grade d motility (%)	29	5–66	12	14	43	47
Normal forms (%)	49	21–72	27	32	59	63
Concentration of peroxidase-negative cells (million/ml)	2.0	1–14	1.0	1.0	6.5	8.0
Peroxidase negative cells per 100 spermatozoa	2.0	0.8–8.5	0.8	0.8	5.5	6.0
Viability (% live)	78	40–92	45	57	89	91
ATP (μmol/liter)	9.2	2.0–54.8	3.0	4.4	34.0	39.0
ATP/million spermatozoa (pmol)	90	40–340	50	60	180	240

[a] Fifth, tenth, ninetieth, and ninety-fifth percentiles.

Table IV. Description of Sperm Characteristics of Semen of Subfertile Men Who Achieved Pregnancy Within 12 Months After Initial Consultation (Median, Range, and Percentile) (9)

	Median	Range	5 P[a]	10 P[a]	90 P[a]	95 P[a]
Sperm concentration (million/ml)	31.5	2.4–163	2.8	4.6	104.5	127.4
Grade a motility (%)	27	3–65	3	6	44	48
Grade a motile sperm concentration (million/ml)	8.00	0.12–81.25	0.23	0.39	31.09	46.11
Grades a + b motility (%)	49	10–87	12	19	67	73
Grades a + b motile concentration (million/ml)	15.68	0.24–108.75	0.42	1.67	48.96	70.56
Grade d motility (%)	40	10–88	16	23	67	80
Normal forms (%)	24	5–60	5	9	39	42
Concentration of peroxidase-negative cells (million/ml)	1.4	0.1–7.0	0.1	0.3	3.5	4.4
Peroxidase-negative cells per 100 spermatozoa	3.5	0.0–26.0	0.1	0.8	21.5	25.8

[a] Fifth, tenth, ninetieth, and ninety-fifth percentiles.

the in vitro results refer to only one cycle of trials, against up to 12 cycles in the in vivo group.

Data on a large group of couples who were systematically investigated for infertility (21,22) have shown coincidental pathology in the female partner of 57% of men with an abnormal semen quality. Hence, overcoming female factors such as ovulation disturbance, minor tubal pathology, and cervical hostility should improve the fertility of the couple. The latter is indeed achieved by in vitro fertilization.

Our data suggest that, if conventional techniques of sperm preparation are used (23), in vitro fertilization has only a minor role to play in the treatment of couples with male infertility. Better techniques of sperm preparation and selection as well as oocyte insemination may improve the success rate in patients with a poor semen quality (24). There may be some advantage in favor of IVF in cases with poor sperm motility but "reasonable" sperm morphology.

It remains to be evaluated whether the success rate of IVF used for the treatment of male infertility exceeds that of less invasive techniques such as intrauterine or intraperitoneal insemination performed under optimal stimulation and monitoring of ovulation.

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