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The Human Sperm Head: A Key for Successful Fertilization

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ABSTRACT: In order to examine the predictive value of determining the sperm head shape, the acrosomal size, the presence of acrosomal vacuoles, and the challenged acrosome reaction (AR) on the outcome of a standard in vitro fertilization (IVF) program, a prospective study was conducted that included 75 couples undergoing IVF treatment. An assessment of sperm morphology was performed using the Hobson Sperm Tracker (Hobson Tracker Limited, Sheffield, United Kingdom). The assessment of the AR was performed before and after adding pooled undiluted human follicular fluid (FF). The outcome measure was an IVF rate of inseminated oocytes. A positive correlation was found between the fertilization rate (FR%) and the proportion of the sperm with a normal (oval) head shape ($P < .001$), the sperm exhibiting acrosomal vacuoles ($P < .003$), the sperm with a normal acrosomal size (40%–70% of total head area, $P < .025$), and the sperm undergoing AR after adding FF ($P < .001$). Multiple logistic regression analysis revealed that by incorporating the above 4 parameters, the sensitivity of prediction of IVF FR% values was 79%, and the specificity was 93%, with a positive predictive value of 96%. This study shows that the multiparametric assessment of the sperm head is useful in predicting the FR% values of a standard IVF treatment. The automated analysis used in this study is shown to maintain a level of precision and accuracy acceptable for application in a routine semen analysis situation.

Key words: Fertilization rates, in vitro fertilization, computer-assisted sperm analysis, acrosome, vacuoles, sperm morphology.

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The prediction of successful fertilization in vitro remains one of the major challenges in the field of assisted conception. Routine semen analysis, which includes an assessment of sperm concentration, motility, and morphology, gives little idea about the patient's ability to achieve successful fertilization in vitro (Barlow et al, 1991). It has been shown that a high percentage of morphologically abnormal sperm is associated with an impaired fertilization rate (FR%) in in vitro fertilization (IVF) (Kruger et al, 1986; Enginsu et al, 1991; Aziz et al, 1996).

The diagnosis of defective sperm function has been facilitated by the recent improvement of diagnostic tests, including computer-assisted sperm analysis (CASA) of sperm morphology (Kruger et al, 1988, 1996), and sperm movement characteristics, including hyperactivation (Burkman, 1991). The development of automated sperm analysis helps to achieve objectivity and to obtain accurate unbiased results (Wang et al, 1991b; Davis and Gravance, 1994). Sperm migration testing (SMT) was previously described as the most useful bioassay in identifying groups of patients likely to achieve fertilization and pregnancy in IVF and intrauterine insemination programs (Biljan et al, 1994; Buckett et al, 1998). Acrosome dysfunction assessed through either the spontaneous acrosome reaction (AR) or the response to inducers such as calcium ionophore A23187 (Fenichel et al, 1991), progesterone (Tesarik and Mendoza, 1992), or phorbol ester (Parinaud et al, 1995) has been shown to discriminate between IVF successes and failures (Aitken and Brindle, 1993; Bronson et al, 1999). Sperm–zona pellucida binding (Morales et al, 1999), zona-free hamster egg penetration test (Shabihara et al, 1998), and assessment of sperm capacitation (Wang et al, 1991a) are other bioassays that are useful in predicting male fertility potential in IVF.

Data from a recent meta-analysis showed that the sperm–zona pellucida binding and the induced AR assays have high predictive power for fertilization outcome. On the other hand, these findings indicated a real need for standardization and further investigation into the potential clinical utility of CASA systems (Oehninger et al, 2000).

The present study aimed to appraise various morphological and functional aspects of the sperm head to determine whether the assessment of these parameters would have a significant predictive value of fertilization in the IVF program.

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Materials and Methods

Patients

Couples undergoing IVF treatment in the Reproductive Medicine Unit, Liverpool Women's Hospital, United Kingdom, between January 2000 and January 2001 were invited to participate in this study. Local ethical committee approval was obtained. Female partners who were older than 40 years or those with a history of severe endometriosis or polycystic ovarian syndrome were excluded. Treatment cycles that resulted in an unfavorable ovarian response (<2 mature oocytes collected) were also excluded from the study. We included in this study male partners who had an SMT value greater than or equal to 5 million/mL, a test used in our unit to decide between a traditional IVF cycle, or intracytoplasmic sperm injection, performed in a previous visit (Biljan et al, 1994).

Methods

Conventional Semen Analysis—Prior to the sperm preparation for the IVF procedure, a standard semen analysis was performed. Semen samples were obtained by masturbation after 3 days of sexual abstinence and allowed to liquefy for 30 minutes at room temperature. Semen volume, sperm count, motility, and morphology were assessed following World Health Organization (WHO) guidelines (1999).

Semen Preparation and SMT—An aliquot of 0.5 mL of semen was put into the bottom of a falcon 2003 tube (Becton Dickinson, Oxford, United Kingdom), with care taken not to contaminate the inner surface of the upper part of the tube. With extreme care, 0.5 mL of medium, IVF-500, Scandinavian (IVF Science AB, Gothenburg, Sweden), was layered over the semen forming a distinct and uniform interface (Biljan et al, 1994). The preparation was kept vertically in an incubator at 37°C for 90 minutes with an atmosphere of 5% CO_2 in air. The uppermost 0.25 mL of the preparation was separated, put in an Eppendorf tube, and mixed. This fraction was divided into 2 parts: the first part was used for sperm migration assessment (motility and concentration), while the second part was utilized for testing the integrity of the AR.

Slide Preparation—Thin smears of semen were prepared by a feathering technique (WHO, 1999) on poly-L lysine-coated slides and stained with the Spermac stain (Stain Interprise, Willington, Republic of South Africa) as previously described (Chan et al, 1999). The slides were mounted, examined, and then kept in a dark cool environment (refrigerator).

Computer-Aided Sperm Morphology Analysis—The Hobson Sperm Tracker (Hobson Tracker Limited, Sheffield, United Kingdom) was used for detailed sperm morphology assessment. A standardized setting of the Hobson Sperm Tracker was maintained throughout the study. We tried different parameters at the start of this work and decided on the settings that follow after a consultation with Prof G. S. Hobson (personal communication). The criteria for a normal sperm head were as follows: head length was 4 to 5 μm, width was 2.5 to 3.5 μm, length-width ratio was 1.5 to 1.75, and acrosomal size was 40% to 70% of total sperm head area (WHO, 1999).

Image recognition was optimized by setting a low numerical aperture on the microscope. This was achieved by opening the condenser iris diaphragm to the point where the defraction halo, around a sperm head, is about to disappear. In the meantime, the microscope phase was changed to phase 0 to give a better image. A green filter was used to enhance image contrast, and the illumination was set at a constant intensity.

For each patient, 100 sperm were analyzed in each slide. After the capture of an image, the CASA system created an image histogram, which displayed the brightness of individual pixels across the image analysis window in relation to their frequency. The Hobson Sperm Tracker produced an outline of blue dots around the captured sperm heads to allow the operator to check the set-up parameters against image recognition. The machine also traced the tail of spermatozoa, thus allowing researchers to ensure that the entire tail was recognized.

The analysis of sperm morphology included the assessment of sperm head size, shape, covering acrosomal area (acrosomal index [AI]), and acrosomal vacuoles. The AI of the sample was defined as the percentage of sperm (out of 100) that exhibited a normal-sized acrosome (40%–70% of the sperm head size). The presence of the acrosomal vacuoles was also confirmed by visual examination on the image screen. This analysis included only those vacuoles that were strictly confined to the acrosome between the outer and inner acrosomal membranes in cells with a normal-looking nucleus.

AR Assay—Assessment of the acrosomal status in viable sperm was performed before (sample A) and after adding undiluted nonheat-treated human follicular fluid (FF) (sample B). Human FF was collected, by aspiration, at the time of oocyte retrieval from the mature follicles (18–20 mm in size) of patients receiving ovulation induction while undergoing IVF. The FF collected was centrifuged at 400 × *g* for 10 minutes to remove any cellular debris (eg, red and white blood cells). The samples were frozen at −20°C until a decision was made whether or not to include them in the study. All FF samples obtained from women who had a high FR% in the same cycle of collection (≥60% fertilization; range, 60–100) were thawed at room temperature, pooled, and thoroughly mixed. Small aliquots of 0.5 mL were prepared and stored again at −20°C. Just prior to use, an aliquot of pooled FF was allowed to thaw at room temperature and was used undiluted (Yao et al, 1999).

The AR was determined by using FITC-PSA (lectin-fluorescein isothiocyanate–pisum sativum agglutinin, Sigma Chemicals, Poole, Dorset, United Kingdom) as previously described (Calogero et al, 1999).

The slides were examined using the green filter of an Axioplan 2 fluorescent microscope, model 2000, with a 100-W HB0 lamp (Zeiss Company Limited, Oberkochen, Germany), which was attached to an Apple Macintosh G3 computer with inbuilt Mac Probe program version 4.2, where the captured image is digitized and analyzed. The AR was evaluated according to the criteria published by Mortimer et al (1987).

Induction of Ovulation and Oocyte Pickup—For female partners, a standard ovarian stimulation long protocol with gonadotrophin-releasing hormone analog, buserelin acetate (Suprefact, Shire Pharmaceutical Ltd, Hants, United Kingdom), and human menopausal gonadotropin (Menogon, Ferring Pharmaceutical UK, Berks, United Kingdom) as previously described (Kingsland et al, 1992) was used. Five thousand international units of

Table 1. *Clinical characteristics and semen parameters (WHO, 1999) in the good and poor IVF fertilizer groups**

Parameter	Good Fertilizers (n = 37)	Poor Fertilizers (n = 38)	Significance (2-tailed) [95% CI]
Female age (y)	33.82	34.84	.44 [−3.6, 1.6]
Male age (y)	33.27	36.05	.053 [−5.6, 2.7]
Duration of infertility (y)	6.31	7.33	.45 [−3.7, 1.6]
Semen volume (mL)	3.67	3.51	.076 [−0.9, 1.2]
Sperm concentration (10^6/mL)	98.56	99.08	.98 [−32, 32]
Sperm motility (%):			
Grade A	38	39	.16 [−12, 2]
Grade B	18	17	.77 [−6, 8.5]
Grade C	8	6	.14 [−0.8, 5.6]
Grade D	38	36	.59 [−5, 8.7]
Normal morphology (%)	33.52	31.5	.48 [−4, 8]

* CI indicates confidence interval; IVF, in vitro fertilization; and WHO, World Health Organization.

human chorionic gonadotrophin (hCG) (Profasi, Serono Laboratories, Welwyn, United Kingdom) was administered by deep intramuscular injection 35 to 36 hours before ultrasound-directed vaginal oocyte retrieval.

Fertilization, Embryo Culture, and Embryo Transfer—Six hours after collection, oocytes were inseminated with 100 000 motile prepared sperm. Oocytes were assessed for fertilization 56 to 58 hours after hCG. The fertilization was detected by determining whether pronuclei were absent or present and by determining the number of pronuclei present, if any. Twenty-four hours later, the fertilized oocytes were examined for cell division, and the resulting embryos were graded according to cell number and morphology. A maximum of 2 embryos were replaced. (The policy of our unit is to transfer 2 embryos at any one time.) The highest-grade embryos were chosen and transferred. Luteal support, in the form of progesterone vaginal pessaries (Cyclogest, Shire), was administered for 2 weeks. Clinical pregnancy was defined by the presence of fetal heartbeats in ultrasound scanning 8 weeks following the transfer.

Statistical Methods—Data were analyzed with inbuilt procedures within the Statistical Package for Social Sciences (SPSS version 10). For all morphological measurements as well as the acrosome analysis, we used the mean percentage of the sperm plus or minus the standard deviation as summary statistics. A 2-sided *t* test was used for comparing results between the means of the studied groups. A *P* value less than or equal to .05 was considered significant. The Spearman rank correlation coefficient (*r*) was used to correlate the outcome of the FR% with various parameters. The chi-square test was used to compare the pregnancy rates in good and poor fertilizer IVF groups. We used multiple logistic regression in building a predictive model of the probability of fertilization given these sperm head parameters.

Results

Seventy-five couples underwent 1 treatment cycle of IVF each, 37 of whom (49.3%) achieved an FR% greater than or equal to 50% (group I, good fertilizers) and 38 of whom (50.7%) achieved an FR% less than 50%, including failed fertilization (group II, poor fertilizers). The mean FR% for group I was 71.3% plus or minus 16.7%, and that for group II was 24.3% plus or minus 14.9% ($P < .001$, confidence interval [CI]: 38.6–55.4).

The mean age of the female and the male partners and the duration of infertility were not significantly different in either group. Sperm count, motility, and sperm morphology, assessed according to WHO criteria (1999), were not significantly different between good and poor fertilizers (Table 1). The mean number of oocytes collected was not statistically different between the 2 groups (6.8 in good and 6.1 in poor fertilizers, $P = .58$).

Influence of Sperm Head Shape and Size on Fertilization

There was a significant difference ($P < .001$, CI: 7, 13.7) in the mean percentage of sperm with a normal head shape (±SD) in group I (29.2% ± 6.7%) when compared with that in group II (18.7% ± 5.7%). The mean percentages of sperm with rounded heads in group I and group II were 33.7% and 41.7%, respectively ($P = .034$, CI: −16, 0.17). Moreover, there was a statistical difference in the percentage of sperm with amorphous head shapes in both groups, the values of which were 3.5% in group I and 5% in group II ($P = .039$, CI: −3, −0.09; Table 2). The FR% correlated positively with the percentage of sperm having a normal (oval) head shape ($r = 0.696$, $P < .001$; Figure, A) and negatively with the percentage of sperm having either a rounded or an amorphous head shape ($r = -0.258$, $P = .047$ and $r = -0.335$, $P = .009$, respectively). No significant correlation was found between the percentage of sperm with macro- or microheads and the FR%.

Influence of Sperm Acrosomal Area and Vacuoles on Fertilization

The mean percentage of sperm with normal acrosomal sizes (AI) in the good fertilizer group was 48.7% plus or

Table 2. *CASA sperm head shape breakdown in the good and poor IVE fertilizer group†*

Parameter	Good Fertilizers (n = 37), Mean % (± SD)	Poor Fertilizers (n = 38) Mean % (± SD)	Significance [95% CI]
Normal (oval)	29.2 (6.7)	18.7 (5.7)	<.001** [7, 13.7]
Borderline	15.4 (4.4)	14.7 (4.9)	.430 [−1.7, 3]
Rounded	33.7 (12.7)	41.7 (18.7)	.034* [−16, 0.17]
Tapered	7.2 (3.7)	6.6 (4.2)	.569 [−1.5, 2.7]
Elongated	11 (8.3)	13.3 (11.6)	.831 [−7.5, 2.8]
Amorphous	3.5 (2.9)	5 (3)	.039* [−3, −0.09]

† CASA indicates computer-assisted sperm analysis; CI, confidence interval; IVF, in vitro fertilization; and SD standard deviation.
* Test is significant at $P = .05$ (2-tailed).
** Test is significant at $P = .001$ (2-tailed).

minus 16.3%, while that in the poor fertilizer group was 38.7% plus or minus 20.3%. This difference was statistically significant ($P = .041$, CI: 0.4, 19.5). The percentage of sperm exhibiting 1 or more acrosomal vacuoles in group I was 72% plus or minus 13.3% in comparison with 61.7% plus or minus 17.9% in group II, and the difference was statistically significant ($P = .013$, CI: 2, 18; Table 3). A significant positive correlation was found between the FR% and the percentage of sperm exhibiting both normal acrosomal size (AI) ($r = 0.289$, $P = .025$; Figure, B) and acrosomal vacuoles ($r = 0.377$, $P = .003$; Figure, C).

Influence of Challenged AR on Fertilization

A significant difference was found between the mean percentage of sperm with spontaneous AR in group I (11.6% ± 5.6%) and group II (27.3% ± 12.5%) ($P < .001$, CI: −21, −10). On the other hand, the mean percentage of sperm that underwent challenged AR was 15.8% plus or minus 6.9% in group I compared with 3.9% plus or minus 2% in group II, and the difference was also statistically highly significant ($P < .001$, CI: 8.5, 15; Table 3). The percentage of sperm undergoing the stimulated AR correlated positively with the FR% ($r = 0.855$, $P < .001$). For the whole study population, a challenged AR correlated positively with the proportion of sperm with oval head shapes ($r = 0.597$, $P < .001$) and negatively with those with amorphous shapes ($r = -0.286$, $P = .05$).

Pregnancy Rates

The overall clinical pregnancy rate was 22.7%. Clinical pregnancy rate negatively correlated with increasing male and female ages ($r = -0.386$, $P = .018$ and $r = -0.34$, $P = .03$, respectively). In our cohort, the duration of infertility prior to IVF treatment had no significant effect on the pregnancy rate.

Among all parameters assessed in this study, the clinical pregnancy rate was positively correlated with the number of fertilized oocytes ($r = 0.292$, $P = .044$) and the proportion of normal sperm assessed by CASA ($r = 0.429$, $P = .003$). The increase in the proportion of sperm with microheads (CASA) had resulted in a decrease in the likelihood of conception ($r = -0.337$, $P = .02$).

Of a total of 37 good fertilizers, 13 (35.1%) achieved clinical pregnancy, whereas only 4 (10.5%) of 38 female partners conceived following the IVF treatment in the poor fertilizers (Table 4). The difference in clinical pregnancy rate in both groups was statistically significant (chi-square test, $P = .038$).

Logistic Regression Model

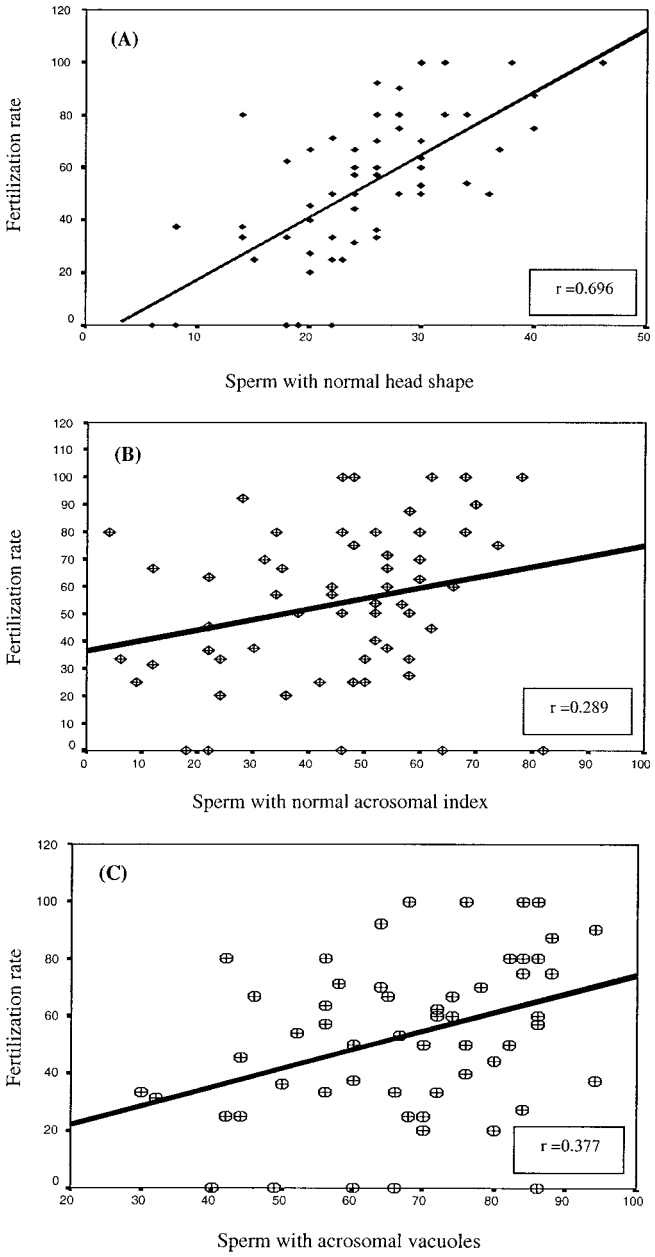
In order to build a model to predict the FR%, all data sets were submitted for logistic regression analysis using a forward stepwise method. The resulting model included the percentage of sperm with normal (oval) head shapes, the percentage of sperm with normal acrosomal sizes, the percentage of sperm with acrosomal vacuoles, and the percentage of sperm with a high acrosomal response to FF.

The actual equation is as follows: logit (P) = Ln [P/(1 − P)] = −2.947 + 0.052 (the percentage of sperm with normal head shapes) − 0.016 (the percentage of sperm with a normal acrosomal size, AI) + 0.025 (the percentage of sperm with acrosomal vacuoles) + 0.065 (the percentage of sperm with a challenged AR), where P is the estimated proportion of successful fertilization, and Ln is the natural log.

In this model, the sensitivity and the specificity of prediction of the successful fertilization were 79% and 93%, respectively. The positive predictive value was 96% with a correct classification rate of 84%.

Discussion

In this study of patients undergoing standard IVF treatment, we demonstrated a significantly positive correlation between the mean percentage of sperm with normal (oval) head shapes, assessed by CASA, and the FR%. These results are in agreement with the findings of Aziz et al (1998), who showed appreciably large proportions of morphologically normal sperm in the semen of all pa-



A scattergram plotted for the fertilization rate (y-axis). **(A)** The mean percentage of sperm with normal (oval) head shapes (x-axis) ($r = 0.696$, $P < .001$). **(B)** The mean percentage of sperm with a normal acrosomal index (AI) (x-axis) ($r = 0.289$, $P = .025$). **(C)** The mean percentage of sperm with acrosomal vacuoles ($r = 0.377$, $P = .003$). *r* indicates correlation coefficient.

tients who achieved fertilization, although the latter study did not use CASA for sperm morphology assessment. Moreover, Marnet et al (2000) demonstrated that a combined sperm morphology and motility assessment by the computer allowed the discrimination of 2 groups of patients with significantly different FR% values. In the current study, a negative correlation was observed between the mean percentage of sperm with both rounded and amorphous head shapes and fertilization. Sperm morphology has been suggested to be a useful predictor of the cell's fertilizing capacity independent of other parameters (Grow et al, 1994; Ombelet et al, 1994). A number of studies have shown that sperm morphology has a limited value in predicting the IVF FR% values (Bartoov et al, 1993; Robinson et al, 1994). However, it must be pointed that these different conclusions may have resulted from differences in staining techniques, sperm preparation protocols, morphological classification systems, and inter- and intraobserver variations in morphology assessment (Menkveld et al, 1990).

By eliminating the human evaluation biases through the standardized quantitative analysis (CASA) in our study, it was feasible to investigate the true association between sperm head morphology and successful fertilization.

In this study, no significant differences were found between the good and the poor fertilization groups with respect to sperm head morphometric measurements. These data confirm our previous findings that the median values of sperm head morphometric features in semen were inefficient in the discrimination between the 2 outcome groups in the IVF treatment (Aziz et al, 1998).

Patients who achieved good fertilization had significantly larger proportions of sperm with normal acrosomal size (area) when compared with poor fertilizers. Moreover, a strong positive correlation was found between the proportion of sperm with a normal acrosomal size and FR%. Jeulin et al (1986) studied semen samples of 17 patients 3 months after their IVF treatment and found that a poor treatment outcome (<33% cleaved embryos) in 8 patients significantly correlated with acrosome defects in semen. No fertilization occurred when the proportion of sperm with abnormal acrosomes was greater than 30%. Menkveld et al (1996) studied the acrosome morphology in 33 patients undergoing IVF treatment and found that all patients with normal FR% values (>50%) had more than 10% of sperm with normal acrosomes. This cutoff point of 10% achieved 100% sensitivity and specificity in their study population. In subsequent work, Menkveld et al (1998) identified that the AI was more sensitive in predicting oocyte fertilization in vitro than the strict normal sperm morphology. The chosen cutoff value of normal acrosome morphology at 10% was remarkably lower than that of the current work and that described by Jeulin et al (1986). Nevertheless, the results of the current work substantiate the available evidence regarding the importance of acrosome morphology in predicting the outcome of IVF. In contrast, Soderlund and Lundin (2001) reported that evaluation of the AI did not accurately predict fertilization. It is possible that the compromised fertilizing ability of sperm is not merely due to abnormal acrosomal morphology, because there is evidence linking acrosome

Table 3. *Acrosomal morphology and spontaneous and induced acrosome reaction in good and poor I'VE fertilizers†*

Parameter	Good Fertilizers (n = 37) Mean % (±SD)	Poor Fertilizers (n = 38) Mean % (±SD)	Significance (2-tailed) [95% CI]
% Sperm with normal acrosomal size (AI)	48.7 (16.3)	38.7 (20.3)	.041* [0.4, 19.5]
% Sperm with acrosomal vacuoles	72 (13.3)	61.7 (17.9)	.013* [2, 18]
Spontaneous acrosome-reacted sperm	11.6 (5.6)	27.3 (12.5)	<.001** [−21, −10]
Induced acrosome-reacted sperm	15.8 (6.9)	3.9 (2)	<.001** [8.5, 15]

† AI indicates acrosomal index; CI, confidence interval; IVF, in vitro fertilization; and SD, standard deviation.
* Test is significant at $P = .05$ (2-tailed).
** Test is significant at $P = .001$ (2-tailed).

defects to sperm nuclear abnormalities and immaturity (Jeulin et al, 1986; Zamboni, 1987).

In the current work, the increase in the mean percentage of sperm with acrosomal vacuoles, when examined by CASA, significantly correlated with the success of fertilization in the IVF program. To our knowledge, this is the first report to demonstrate the significance of acrosomal vacuoles as a marker of successful fertilization. Unlike vacuolated sperm heads, these vacuoles were concentrated between the inner and outer acrosomal membranes. It has been shown that the migration of limited amounts of acrosin to the sperm surface is the earliest event characterizing the beginning of the AR. The acrosome of such spermatozoa remained morphologically intact or swollen, or it showed intra-acrosomal vesiculation without any disruption of the plasma membrane and acrosomal membrane (Tesarik et al, 1988).

Along with previous studies (De Jonge et al, 1993; Yao et al, 1999), our study shows that human FF induces the AR. The challenged AR in the current work was more pronounced in the good fertilizer group than in the poor fertilizer group.

In our cohort, the increase in the proportion of sperm with specific head shapes had an influential effect on the rates of AR, with the rate being positively correlated to those sperm with normal (oval) heads. These findings are in agreement with the results of Fukuda et al (1989), who reported that morphologically abnormal sperm had lower rates of acrosomal loss after incubation with FF than normal sperm.

There is evidence from this study to suggest that pregnancy was dependent on the proportion of normal sperm morphology in semen. Bianchi et al (1996) studied the influence of sperm with normal morphology and their chromatin packaging on FR% values, embryo cleavage, and pregnancy. They concluded that normal sperm morphology correlated with fertilization but was unrelated to embryo cleavage and pregnancy rates. Other groups, however, could not identify a significant correlation between normal sperm morphology in semen and pregnancy (Check et al, 1992; Figueiredo et al, 1996). In a meta-analysis of published data between 1988 and 1996 on the prediction of pregnancy rate per cycle using sperm morphology, Coetzee et al (1998) concluded that overall pregnancy rates increased with an increase in the proportion of sperm with a normal morphology, supporting the findings of our study.

In conclusion, we present, for the first time, compelling evidence that the fertilization outcome of an IVF program can be predicted on the basis of the CASA assessment of sperm head morphology and the AR. The proposed model for predicting good and poor fertilization had a high sensitivity and specificity. This could have important implications in identifying those couples likely to require microassisted fertilization techniques.

Table 4. *Clinical pregnancy rates in the good and poor IVF fertilizer*

Group	Clinical Pregnancy No	Clinical Pregnancy Yes	Total
Good fertilizers	24	13	37
Poor fertilizers	34	4	38
Total	58	17	75

* IVF indicates in vitro fertilization.

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