Prediction of outcomes of assisted reproduction treatment using the calcium ionophore-induced acrosome reaction

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BACKGROUND: Sperm concentration and motility are poor predictors of the outcome of intrauterine insemination (IUI), hysteroscopic intratubal insemination (HIT), or complete fertilization failure (CFF) in conventional IVF. We investigated whether the calcium ionophore-induced acrosome reaction (AR) constitutes an additional indicator of CFF and pregnancy that is independent of these semen parameters. METHODS: Infertile couples with no female factor (n = 388) and women with tubal obstruction (n = 32) were studied: IVF (n = 133), ICSI (n = 72), HIT (n = 245) and IUI (n = 61). The percentage of acrosome-reacted sperm in relation to viable sperm was calculated. Receiver operating characteristic curve and multiple logistic regression analyses were used to determine threshold values and the best predictor for CFF and pregnancy. RESULTS: Threshold values of AR for predicting CFF in IVF and pregnancy in IVF and HIT + IUI were 21, 26 and 22% respectively. These values were independent of the conventional semen analysis parameters. CFF was lower (2 versus 20%; P < 0.01) and the pregnancy rate was higher (46 versus 24% P < 0.05) for those with AR >21% in IVF. CFF and pregnancy rate in ICSI did not differ according to AR. Pregnancy rate was higher for those with an AR >22% for HIT + IUI (23 versus 11% P < 0.01). CONCLUSIONS: Ionophore-induced AR appears to be a useful indicator in addition to routine semen analysis for selection of patients for treatment with appropriate assisted reproduction procedure.

Key words: acrosome reaction/assisted reproductive technology outcomes/calcium ionophore/complete fertilization failure

Introduction

Prediction of the outcome (fertilization and pregnancy) of assisted reproductive treatment is important in order to assign patients for different treatment procedures. Although one of the most important factors determining the treatment procedure for individual couples undergoing assisted reproduction is sperm quality, we often experience low fertilization rates after conventional IVF, despite normozoospermia, as defined by the World Health Organization (WHO, 1999). The low fertilization rate, particularly complete fertilization failure (CFF), after IVF is frustrating and has serious psychological and economic effects on patients. Conversely, the CFF rate in ICSI is reported to be much lower than that in IVF: 3 versus 10–20% respectively (Lipitz et al., 1994; Liu et al., 1995). Worldwide, a number of groups have abandoned IVF and use ICSI as the standard fertilization procedure, even when the sperm parameters are normal (Devroey and Van Steirteghem, 2004). However, if this change continues to be adopted without further studies being undertaken, it could have serious effects on laboratory and medical resources (Oehninger, 2001). Moreover, because ICSI is a fairly new technique, there has been insufficient follow-up with respect to the children born after ICSI, and with respect to its overall safety, despite the fact that it bypasses the natural mechanism of gamete selection and constitutes an invasive technique (Oehninger, 2001). De novo sex chromosomal aneuplody, structural autosomal abnormalities, and inherited aberrations are all reported to be slightly, but significantly, increased in children born after ICSI (te Velde et al., 1998; Van Steirteghem et al., 2002), although the incidence of major malformations is 3–4%, which is within the range observed in the general population (Van Steirteghem et al., 1998, 2002; Palermo et al., 2000; Bonduelle et al., 2002). The efficacy of ICSI in borderline or normozoospermia remains controversial. A large randomized controlled trial with non-male factor subfertility showed recently that the implantation and pregnancy rates were higher in IVF than in ICSI (Bhattacharya et al., 2001; Van Steirteghem and Collins, 2003). Rhemrev et al. (2001) concluded that ICSI offers no advantage over IVF in terms of clinical outcome in non-male factor subfertility. ICSI should only be proposed where there is an unacceptably high chance of CFF (Rhemrev et al., 2001). Unfortunately, criteria have not been established for choosing the mode of assisted
reproduction for treating patients with non-severe male factor using conventional semen analysis for predicting the outcome: intrauterine insemination (IUI), hysteroscopic insemination into tube (HIT), IVF or ICSI. A morphological assessment using ‘strict criteria’ for normal sperm morphology is one method that appears to predict the outcome of assisted reproduction (Grow and Oehninger, 1995; Ombelet et al., 1995). However, the validity of the strict criteria routinely used in laboratories remains controversial (Rhemrev et al., 2001). We have reported that the ionophore-induced acrosome reaction (AR) also predicts sperm fertilizing ability in the hamster test relatively accurately (Kumagai, 1994). As a sperm functional test, the ionophore-induced AR may predict the capacity of sperm to accomplish fertilization and pregnancy (Yovich et al., 1994; Liu and Baker, 2000; Soderlund and Lundin, 2001).

In this study we investigated whether the calcium ionophore-induced AR is an additional indicator, independent of conventional semen parameters (range, sperm concentration >20 ×10^6/ml and motility >50% WHO, 1999), for CFF in IVF and pregnancy in IVF and HIT + IUI, in order to determine the best choice of assisted reproduction, using receiver operating characteristic (ROC) curve and multiple logistic regression analyses.

Materials and methods

Patients

Four hundred and twenty infertile couples undergoing treatment at Hiroshima University Medical Center between April 1997 and February 2002 participated in this study. During the infertility work-up, we followed a standard protocol for history-taking, physical examination, and investigations for all patients, including conventional semen analysis on at least two occasions, basal body temperature, hysterosalpingography, serum progesterone, prolactin, thyroid-stimulating hormone in the luteal phase, post-coital test, and transvaginal ultrasonography. In this study, those couples undergoing IUI and HIT had: (i) no female factor, (ii) no ovarian stimulation except for clomiphene citrate, and (iii) total motile sperm ≥1 ×10^6/ml at work-up and insemination; those undergoing IVF had: (i) no female factor with or without tubal patency, and (ii) a semen concentration ≥1 ×10^6/ml and motility ≥20% at work-up and insemination; and those for ICSI had: (i) no female factor with or without tubal patency and (ii) no microsurgical sperm aspiration (MESA) or testicular sperm extraction (TESE) for semen collection. There were 388 couples with no female factor for infertility, while in another 32 couples; tubal obstruction was demonstrated on hysterosalpingography (HSG) or laparoscopy. IVF, ICSI, IUI and HIT were used in 133, 72, 61 and 245 couples, respectively. Some couples with unsuccessful outcomes using one procedure underwent treatment with another assisted reproduction treatment.

Semen analysis and ionophore A23187-induced AR

Testing for an ionophore-induced AR was performed as one of the routine investigations during the infertility work-up. A semen sample was obtained by masturbation after 5 days of abstinence. Liquefaction, initiated within 30 min of collection, was achieved by incubation in a 15 ml test tube at 37°C in an atmosphere including 5% CO₂. Sperm numbers and motility were determined by a visual estimation using a Makler counting chamber (SEFI Medical, Israel).

We measured a combination of capacitation and A23187-induced AR, because sperm with AR after capacitation can fertilize in vivo. Semen was added to modified Biggers–Whitten–Whittingham medium (mBWW) with HEPEPS (0.04 mol/l) and 0.5% (w/v) human serum albumin (HSA, fraction V; Sigma Chemical, USA) to give a total volume of 10 ml. After two 5 min centrifugation in this medium at 200 g, the pellet was gently deposited at the bottom of a test tube, and 1 ml of HSA–mBWW medium was layered above it. Motile sperm were allowed to swim up into the medium for 1 h. They were then pipetted up from the medium and incubated for an additional 5 h. A 1 mmol/l calcium ionophore A233187 (Sigma Chemical) stock solution in dimethylsulphoxide (DMSO; Sigma Chemical) was prepared and 10 μl aliquots were frozen at −20°C. Before use, this was thawed, dissolved in 0.5 ml of HSA–mBWW, and added to 0.5 ml of the motile sperm suspension (2 ×10^6/ml) to make a final concentration of 10 μmol/l. Incubation was continued for 1 h. The sperm were then centrifuged at 200 g for 5 min. To assess viability, staining also was performed with 1 μg/ml Hoechst 33258 (Sigma–Aldrich, USA) in 0.5 ml of HSA–mBWW for 7 min. The sperm were then centrifuged again at 200 g for 5 min, spread over the entire area of a glass slide, air-dried in a dark room, and fixed in 100% ethanol for ≥30 min. On removal from the ethanol, the slide was stained with 100 μg/ml of fluorescent isothiocyanate-conjugated Pisum sativum agglutinin (FITC–PSA; FL1051, Vector Laboratories, USA) for 10 min. The slide was washed in distilled water, dried, treated with an anti-fading agent (15 pg; Bio-Rad, USA), and a coverslip was put on it. The edges were sealed with tape.

Acrosome assessment

We used an epifluorescence microscope (X2F-EFD2; Nikon, Japan) with two filters to observe fluorescence from the Hoechst 33258 (∼100, UV filter) and FITC–PSA (∼100, B2 filter). Sperm were considered non-viable when they showed strong blue-white fluorescence behind the centre of the head with Hoechst 33258; the heads of viable sperm were unstained or stained only lightly. When more than half of the head of a spermatozoon fluoresced brightly and uniformly with FITC–PSA, the acrosome was considered intact (Liu and Baker, 1998). Sperm without fluorescence or with a fluorescing band limited to the equatorial segment were considered acrosome-reacted. Of these acrosome-reacted sperm, viable sperm with a fluorescing band limited to the equatorial segment were counted to determine the percentage of AR. The AR was calculated as the number of viable sperm that stained strongly in the equatorial segment divided by the number of viable sperm (Kumagai, 1994). At least 300 sperm per sample were examined to determine the AR. One examiner performed the experiment and assessment at the same time and temperature, and under the same atmospheric conditions. Intra-individual variation was confirmed to be acceptable; the mean ± SD and coefficient of variation (CV) of the AR between samples of normal fertile volunteers were 31.3 ±1.9% and 6.1% respectively.

IUI and HIT procedures

Follicular growth was monitored ultrasonographically using a transvaginal probe, and IUI was scheduled to be performed on the day after the largest follicle reached a diameter of 18–20 mm in a natural cycle, or 20–22 mm in a cycle stimulated using clomiphene citrate. The semen sample for insemination was analysed before and after processing for conventional parameters (volume, sperm count, and motility; WHO, 1999). After the semen had been incubated at 37°C for 20 min, PureCeption (Sage Bio Pharma, USA) was used to separate sperm by centrifugation at 300 g for 30 min.
(Claassens et al., 1998). The pellet was washed with 3.0 ml of fresh medium, centrifuged for 5 min at 500 g, and resuspended in a total volume of 0.4 ml for IUI or 50 μl for HIT. Insemination was performed at 38–40 h after hCG (Gonatropin®, Teikokuzoki, Japan) administration. The insemination for IUI was carried out using a simple catheter. For insemination for HIT, a sufficiently slender hysterofibrescope (HYF-P; Olympus; outer and channel diameters, 3.6 and 1.2 mm respectively) was inserted through the cervical canal without dilation. Under hysteroscopic observation, the catheter used for insemination was inserted 0.5–1.0 cm into the oviduct on the side of the dominant follicle. Patients were kept on bed rest for 30 min after IUI and HIT.

Conventional IVF and ICSI procedures

Pituitary secretion was down-regulated by administering 150 μg of buserelin (Supercuro®; Aventis, Japan), a GnRH analogue, as a nasal spray three times daily, beginning in the mid-luteal phase of the cycle preceding the treatment cycle. Pituitary down-regulation was confirmed using both transvaginal scanning and serum estradiol measurement performed on day 7 of the treatment cycle. Injections of hMG (150–300 IU/day; Humegon®; Organon, The Netherlands; or hMG Nikken®; Nikken Kagaku, Japan) were then begun. Follicular growth was monitored using transvaginal scanning and serum estradiol concentrations. An i.m. injection of hCG (10 000 IU) was given when at least two follicles were >17 mm in diameter. Oocyte retrieval was performed 36 h after hCG injection. After each oocyte had been cultured for 4 h in human tubal fluid (HTF; Irvine Scientific, USA), it was inseminated with sperm (IVF) or injected (ICSI). For both methods, the sperm used had ‘swum up’ during 2 h incubation at 37°C with 5% CO₂ in the air. ICSI was performed after the cumulus and corona cells had been removed enzymatically with 80 IU/ml hyaluronidase (H-3757, Sigma–Aldrich). Fertilization was determined by whether two pronuclei could be identified after ~20 h. Fertilized oocytes were cultured for 24 h in 1 ml of medium per ovum before transfer into the uterus (maximum, three oocytes) on day 2 after oocyte retrieval. After embryo transfer, vaginal progesterone (300 mg/day) was used to induce the luteal phase. Pregnancy was detected by demonstrating a gestational sac using transvaginal scanning and serum estradiol concentrations. An i.m. injection of hCG (150–300 IU; Humegon®; Organon, The Netherlands; or hMG Nikken®, Nikken Kagaku, Japan) were then begun. Follicular growth was monitored using transvaginal scanning and serum estradiol concentrations. An i.m. injection of hCG (10 000 IU) was given when at least two follicles were >17 mm in diameter. Oocyte retrieval was performed 36 h after hCG injection. After each oocyte had been cultured for 4 h in human tubal fluid (HTF; Irvine Scientific, USA), it was inseminated with sperm (IVF) or injected (ICSI). For both methods, the sperm used had ‘swum up’ during 2 h incubation at 37°C with 5% CO₂ in the air. ICSI was performed after the cumulus and corona cells had been removed enzymatically with 80 IU/ml hyaluronidase (H-3757, Sigma–Aldrich). Fertilization was determined by whether two pronuclei could be identified after ~20 h. Fertilized oocytes were cultured for 24 h in 1 ml of medium per ovum before transfer into the uterus (maximum, three oocytes) on day 2 after oocyte retrieval. After embryo transfer, vaginal progesterone (300 mg/day) was used to induce the luteal phase. Pregnancy was detected by demonstrating a gestational sac using transvaginal ultrasonography after a positive urine pregnancy test (hCG ≥ 50 IU).

Statistical analysis

The results are presented as the mean ± SD. P < 0.05 was considered significant. The semen parameters, semen count, motility and AR in the CFF and non-CFF groups were compared in the IVF and ICSI groups using the Mann–Whitney U-test, as were pregnancy and non-pregnancy groups in the IVF, ICSI and HIT + IUI groups.

A receiver operating characteristic (ROC) curve analysis was used to determine the threshold values of the AR for predicting CFF in IVF and pregnancy in IVF and HIT + IUI. Multiple logistic regression analyses were performed using the semen parameters, AR threshold values, reference values of the semen analysis, concentration and motility (WHO manual, 1999; 20 × 10⁶/ml and 50%) to predict CFF in IVF and pregnancy in IVF and HIT + IUI. The CFF and pregnancy rates in the IVF and ICSI groups were compared using a threshold of 21% using the χ² distribution, as was the fertilization rate in the IVF and ICSI groups using the Mann–Whitney U-test, with the same threshold of 21%. The pregnancy rates in the IVF and HIT + IUI groups were compared using the threshold value with the χ² distribution.

Results

Background characteristics

The men in the study were 34.1 ± 5.4 (22–56) years old (mean ± SD), and their partners were 32.9 ± 4.1 (22–44) years old. Semen volume was 2.7 ± 3.6 (0.2–8.5) ml; total sperm count was 80.9 ± 70.2 × 10⁷ (0.1–503.0 × 10⁷); percentage of motile sperm was 53.6 ± 21.5% (0.2–96); and the AR was 28.3 ± 17.4% (0–77.5). The duration of failure to conceive was 33.2 ± 26.6 (12–112) months; the interval from initial treatment was 27.8 ± 24.3 (2–92) months.

Semen parameters and assisted reproduction outcomes

Of the 133 cases undergoing IVF and 72 cases undergoing ICSI, 11 (8.3%) and three (4.2%) CFF occurred respectively. The mean semen concentration, motility and AR for CFF and non-CFF in IVF and ICSI are summarized in Table I. Among IVF cases, the AR was significantly lower in the CFF group (P < 0.01). The semen concentration was also significantly lower in the CFF group (P < 0.05), although the mean values in both groups were quite high, compared with the WHO (1999) reference value (20 × 10⁶/ml). Sperm motility in semen was similar in both CFF and non-CFF groups. Among ICSI cases, the AR and sperm motility in semen were similar in both the CFF and non-CFF groups, whereas the semen concentration was significantly lower in the CFF group (P < 0.05). The mean value in the CFF group was quite low, compared with the value in non-CFF: 3 ± 3 × 10⁶ versus 42 ± 48 × 10⁶ respectively.

Of the 420 women, 137 became pregnant. Two patients became pregnant again, by IUI, after pregnancies by IVF or ICSI. There were 51 (38.3%), 30 (41.7%), nine (14.8%) and 47 (19.2%) patients who became pregnant in the IVF, ICSI, IUI and HIT groups respectively. Due to re-treatment after failure, some patients were included in multiple groups. The mean semen concentration, motility and AR for pregnant or non-pregnant in IVF and ICSI are summarized in Table II. Among ICSI cases, semen parameters were similar in both pregnant and non-pregnant cycles. Among IVF cases, the AR was significantly higher in the pregnant group (P < 0.01). The semen concentration was also significantly higher in the pregnant group (P < 0.05), although the mean values in both groups were within the WHO (1999) reference value. Among HIT + IUI cases, the AR was significantly higher in

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Table I. Comparison of results of sperm tests between patients with CFF and non-CFF in IVF and ICSI

<table>
<thead>
<tr>
<th></th>
<th>IVF (n = 133)</th>
<th>ICSI (n = 72)</th>
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<tbody>
<tr>
<td></td>
<td>CFF (n = 11)</td>
<td>Non-CFF (n = 122)</td>
</tr>
<tr>
<td>Concentration (×10⁷)</td>
<td>50 ± 43a</td>
<td>95 ± 76a</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>52 ± 13</td>
<td>57 ± 20</td>
</tr>
<tr>
<td>AR (%)</td>
<td>14 ± 13b</td>
<td>32 ± 18b</td>
</tr>
</tbody>
</table>

aP < 0.05 and bP < 0.01 between CFF and non-CFF.

CFF = complete fertilization failure; AR = acrosome reaction.
the pregnant group ($P < 0.01$). None of 16 couples with an AR <11.3% achieved pregnancy in HIT + IUI, despite a total of 63 IUI. Semen motility was also significantly higher in the pregnant group ($P < 0.05$), although the mean values in both groups were within the WHO (1999) reference value (>50%).

**ROC curve and multiple logistic regression analyses**

The threshold values of the AR for predicting CFF in IVF, pregnancy in IVF, and pregnancy in HIT + IUI determined using ROC curves were 21, 26 and 22% respectively (Figures 1–3). The value of each AR threshold for predicting CFF in IVF and pregnancy in IVF or HIT + IUI are summarized in Table III. The threshold value of the AR for predicting CFF in IVF was significant and independent of the other semen parameters (semen concentration and motility) in the multiple logistic regression analysis (OR $= 0.09$, 95% IC $= 0.02–0.45$, $P < 0.01$; Table IV), as well as pregnancy in IVF or HIT + IUI (IVF; OR $= 2.68$, 95% IC $= 1.23–5.86$, $P < 0.05$, HIT + IUI; OR $= 2.29$, 95% IC $= 1.14–4.60$, $P < 0.05$; Table IV).

The CFF, fertilization and pregnancy rates in the groups undergoing IVF or ICSI with an AR <21% or >21% are summarized in Table V. The CFF rate was significantly higher in IVF couples with an AR <21% ($P < 0.01$). The fertilization and pregnancy rates were lower in IVF couples with an AR <21% (fertilization: $P < 0.01$, pregnancy: $P < 0.05$). The CFF, fertilization and pregnancy rates in ICSI did not differ significantly between couples with an AR >21% or <21%. With an AR <21%, the CFF rate was significantly higher in IVF than in ICSI (20 versus 4%, $P < 0.05$), while the CFF rates were similar in both IVF and ICSI with AR >21%.

The pregnancy rates when the AR was less than or more than the threshold value for couples undergoing IVF and HIT + IUI are summarized in Table VI. The pregnancy rates were significantly lower in both IVF and HIT + IUI couples below the AR threshold value (both $P < 0.01$).

**Discussion**

The AR threshold values determined using ROC curves for predicting CFF and pregnancy in IVF, which were 21 and 26% respectively, were the sole predictors independent of the conventional semen analysis in the multiple logistic regression analysis. Conversely, the conventional semen parameters were unrelated to both CFF and pregnancy. In ICSI, CFF, fertilization and pregnancy were unrelated to the AR. The fertilization rate of those with an AR <21% did not differ in IVF and ICSI, which were 38 versus 42% respectively. By contrast, within this group, the CFF rate in IVF differed significantly from that in ICSI, i.e. 20 versus 4% respectively. The pregnancy rate for this group was lower in IVF than in ICSI, i.e. 24 and 41% respectively, although these values were not significantly different. Conversely,

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**Table II. Comparison of sperm analysis between pregnant and not pregnant in IVF, ICSI and HIT + IUI**

<table>
<thead>
<tr>
<th></th>
<th>IVF (Pregnant)</th>
<th>IVF (Not pregnant)</th>
<th>ICSI (Pregnant)</th>
<th>ICSI (Not pregnant)</th>
<th>HIT + IUI (Pregnant)</th>
<th>HIT + IUI (Not pregnant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (×10⁶)</td>
<td>107 ± 86ᵃ</td>
<td>81 ± 65ᵃ</td>
<td>44 ± 52</td>
<td>37 ± 44</td>
<td>88 ± 68</td>
<td>83 ± 71</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>60 ± 18</td>
<td>54 ± 19</td>
<td>40 ± 21</td>
<td>41 ± 23</td>
<td>60 ± 20ᵇ</td>
<td>53 ± 23ᵇ</td>
</tr>
<tr>
<td>AR (%)</td>
<td>36 ± 19ᵇ</td>
<td>27 ± 17ᵇ</td>
<td>18 ± 3</td>
<td>16 ± 2</td>
<td>35 ± 15ᵇ</td>
<td>28 ± 17ᵇ</td>
</tr>
</tbody>
</table>

ᵃ$P < 0.05$ and ᵃᵇ$P < 0.01$ between pregnant and not pregnant.

HIT = hysteroscopic insemination into the Fallopian tube; IUI = intrauterine insemination; AR = acrosome reaction.

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**Figure 1.** Receiver operating characteristic (ROC) curve used to determine the best predictive value of the percentage acrosome reaction for complete fertilization failure (CFF) in IVF.

**Figure 2.** Receiver operating characteristic (ROC) curve used to determine the best predictive value of the percentage acrosome reaction for pregnancy.
the CFF, fertilization and pregnancy rates of those with an AR > 21% in IVF were not significantly different from those in ICSI, i.e., 2 versus 5%, 65 versus 51%, and 46 versus 43% respectively. These findings indicate that IVF is the best choice to avoid CFF for those with an AR > 21%, without regard to sperm concentration and motility except when there is an absolute indication for ICSI, based on damage to cell structures. Conversely, ICSI is the best choice for avoiding CFF for those with an AR < 21%. If all oocytes were fertilized using ICSI, the number needed to be treated using ICSI in order to prevent CFF after IVF would be too high, as Tournaye (2000) have reported. In our series, four ICSI procedures would have to be performed instead of conventional IVF in couples with an AR < 21% to prevent one CFF; there were nine CFF cases in the 45 cases undergoing IVF with an AR < 21%. Moreover, there is no evidence of a difference between ICSI and IVF for couples with non-male factor sub-fertility with respect to implantation and pregnancy rates (Bhattacharya et al., 2001; Van Steirteghem and Collins, 2003). If anything, the outcome was in favour of IVF, the less invasive method (van Rumste et al., 2004), and the use of ICSI should be proposed only where there is an unacceptably high chance of CFF (Rhemrev et al., 2001). The CFF rate of those with an AR < 21% was relatively high, as compared to those with an AR > 21% in IVF. In order to avoid CFF and obtain fertilized oocytes using IVF, split fertilization should be performed when at least five cumulus–oocyte complexes are available: two for ICSI and three for IVF. Based on the fertilization rate in our unit, 42% in ICSI and 38% in IVF for those with an AR < 21%, an average of one of the two metaphase II oocytes would be fertilized by ICSI and one of the three oocytes would be fertilized by IVF, unless CFF occurs. The split of oocytes between IVF and ICSI should be determined using the IVF and ICSI fertilization rates in each facility.

The threshold AR value determined using the ROC curve for pregnancy in IVF was 26%. The difference in the threshold values for CFF and pregnancy in IVF, 21 and 26% respectively, suggests that other factors affecting embryo quality for implantation and uterine receptivity have an effect on determining the threshold value for pregnancy. The threshold value of the AR, determined using the ROC curve for pregnancy with in vivo insemination, was 22%. The most important factor for determining the outcome of in vivo insemination might be fertilization. The minimum value of the AR for pregnancy with in vivo insemination was 11.3%. This suggests that those with an AR < 10% should not repeat in vivo insemination, but step up to in vitro or split fertilization. In this series, the combined HIT and IUI data were analysed as in vivo insemination data because the AR was not independent of conventional semen parameters in the analysis of each separate treatment procedure. In addition to AR, the total numbers of motile and morphologically normal sperm, numbers of sperm used for insemination, and woman’s age significantly affect the pregnancy rate in IUI (Bielsa et al., 1994; Campana et al., 1996). As for the threshold value for pregnancy with in vivo insemination, more power is needed to verify that the AR is independent of the conventional semen parameters in each modality.

In a previous report, we verified that the percentage of viable sperm with a fluorescing band limited to the equatorial segment was associated with the sperm penetration rate for hamster oocytes and the sperm penetration index more closely than either non-viable sperm with a fluorescing band limited to the equatorial segment or non-fluorescing sperm (Kumagai, 1994). Sperm with a fluorescing band limited to
Baker, 1998). Conversely, Oehninger zoospermia than to specific defects of the AR (Liu and AR might be more closely related to the presence of terato-
lysis to clarify whether the AR is an independent parameter not assessed. We plan to use multiple logistic regression ana-
served. We did not clarify the relationship between AR and using the strict criteria, to a calcium ionophore was con-
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<table>
<thead>
<tr>
<th>Table V. The CFF, fertilization, and pregnancy rate in IVF and ICSI according to percentage of acrosomal reaction (AR)</th>
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<tbody>
<tr>
<td>AR</td>
</tr>
<tr>
<td></td>
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<tr>
<td>&lt;21%</td>
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<td>&gt;21%</td>
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<tr>
<td>Total (%)</td>
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<sup>a</sup>P < 0.01,  
<sup>b</sup>P < 0.05 between <21% and >21%.  
<sup>c</sup>P < 0.05 between IVF and ICSI.  
CFF = complete fertilization failure; AR = acrosomal reaction.

<table>
<thead>
<tr>
<th>Table VI. The pregnancy rate in IVF and HIT + IUI according to AR</th>
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<tr>
<td>AR</td>
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<tr>
<td>Less than threshold value (%)</td>
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<tr>
<td>More than threshold value (%)</td>
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<td>Total (%)</td>
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<sup>a</sup>P < 0.01 between less and more than threshold value.  
Threshold value; 26% for IVF, 22% for HIT + IUI.  
HIT = hysteroscopic insemination into the Fallopian tube; IUI = intrauterine insemination; AR = acrosomal reaction.

Considering sperm morphology evaluated using the strict criteria.

In conclusion, ionophore-induced AR provides an additional indicator, which is independent of conventional semen parameters for prediction of CFF in IVF and pregnancy in IVF and HIT + IUI. Assessment of the ionophore-induced AR in patients before commencing assisted reproduction treatments may improve their clinical management.

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