FERTILITY AND STERILITY
Vol. 61, No. 5, May 1994

Printed on acid-free paper in U. S. A.

Use of the acrosome reaction to ionophore challenge test in managing patients in an assisted reproduction program: a prospective, double-blind, randomized controlled study

Jeanne M. Yovich, B.Sc.
W. Rohini Edirisinghe, Ph.D.
John L. Yovich, M.D.*

PIVET Medical Centre, Perth, Western Australia

Objective: To assess the utility of the acrosome reaction (AR) to ionophore challenge test in determining the sperm treatment protocols for patients undergoing assisted reproduction.

Design, Setting, Patients: One hundred twenty-one couples undergoing an IVF-ET or GIFT procedure from January to July 1992 were included in this prospective study. All cases had a preliminary semen analysis within the previous 3 months and an AR to ionophore challenge test was carried out unless an acceptable fertilization rate occurred on previous IVF. For those patients whose AR to ionophore challenge score was below the accepted fertile range of ≥10%, a second AR to ionophore challenge test was performed after exposure of sperm to the stimulant pentoxifylline. Couples then were managed by assisted reproduction with randomized allocation of oocytes for fertilization with a standard sperm preparation or with added sperm stimulants, either 3.6 mM pentoxifylline alone or combined with 3.0 mM 2-deoxyadenosine. The study was double-blind with neither the patients nor the embryologist knowing the AR to ionophore challenge result at the time of the IVF procedure.

Main Outcome Measures: Data from the preliminary semen analyses and AR to ionophore challenge scores were correlated with the fertilization rates achieved using control and treated sperm preparations. The rates of total fertilization failure and the numbers of clinical pregnancies occurring in each subgroup were also recorded.

Results: All AR to ionophore challenge groups showed normal sperm counts except the groups with poor AR to ionophore challenge, which demonstrated reduced sperm counts. The group with normal AR to ionophore challenge scores or previous normal fertilization showed satisfactory fertilization rates with either control or treated sperm, although some individual cases showed reduced fertilization with treated sperm. The fertilization rate for the group with low AR to ionophore challenge scores improved significantly with pentoxifylline, and the benefit was greatest when this had been predicted from the AR to ionophore challenge studies. Cases with persisting poor AR to ionophore challenge despite pentoxifylline showed no significant improvement in fertilization rates with sperm exposed to either sperm stimulant regimens. Poor AR to ionophore challenge scores were also predictive of total fertilization failure, but this problem was reduced by sperm stimulation. The AR to ionophore challenge score at 10% cutoff level showed optimal levels of sensitivity (82.1%), highest negative predictive value (82.1%), and lowest false negative rate (17.9%).

Conclusions: The AR to ionophore challenge test is useful in the assessment and management of the male factor in assisted reproduction. It can be used to identify the majority of cases who will benefit from the use of sperm stimulants. Fertil Steril 1994;61:902–10

Key Words: ARIC, pentoxifylline, acrosome, 2-deoxyadenosine, sperm, assisted reproduction

Received April 20, 1993; revised and accepted January 14, 1994.

* Reprint requests: John L. Yovich, M.D., PIVET Medical Centre, 166–168 Cambridge Street, Leederville, Perth, 6007, Western Australia, Australia (FAX: 61-9-382-4576).

Over recent years major emphasis has been focused on the male factor in the management of infertility, and it has become increasingly clear that the standard semen analysis has limited predictive value for fertilization. Spermatozoal motility has

been considered as the single most important factor determining fertilization in assisted reproduction, although more recently, strict criteria morphology has also been shown to be relevant (1). With the recent advent of computer-automated semen analysis, good correlations have been made between the fertilization rate and certain motility parameters such as curvilinear velocity and lateral head displacement (2). However, the definitive test system for sperm is IVF of human oocytes, which is properly governed by ethical constraints and cannot be used for a purely diagnostic purpose. The zona-free hamster oocyte penetration assay that is often used for assessing the fertilization potential of spermatozoa has certain limitations (3, 4). Furthermore, it is time-consuming and expensive to run especially in Australia where live hamsters are not allowed, so hamster oocytes imported from overseas are the only possible supply. Alternatively, the acrosome reaction (AR) to ionophore challenge test that studies the acrosomal status of spermatozoa in vitro has been shown to correlate well with fertilization (5). In this test, the difference between the spontaneous reaction rate in a control subpopulation and that seen in a sample stimulated with the ionophore A23187 is estimated. Using an AR to ionophore challenge cutoff at 10%, the sensitivity of the test in predicting subfertility is 54% with a positive predictive value of 64% and specificity of 85%. It was shown that of 53 fertile men studied, none had AR to ionophore challenge values $< 5\%$, a level that provides a 90% positive predictive value in assessing subfertility. However, the higher value ($\geq 10\%$) was chosen for its better sensitivity.

The AR is a prerequisite for fertilization and has been studied extensively by many researchers using different techniques such as the triple stain (6), various lectins with affinities to different components of the acrosome (6–8), and monoclonal antibodies (9). The technique used for assessing the acrosomal status of sperm in the AR to ionophore challenge test is a modification of that described by Cross et al (7). In this technique, the acrosome is visualized using fluorescein-conjugated pisum sativum lectin, which binds to the acrosomal matrix once the plasma and outer acrosomal membranes are permeabilized with ethanol. The aim of this study was to assess the utility of the AR to ionophore challenge test in determining the appropriate treatment protocols for sperm preparation when managing patients in an assisted reproductive program.

MATERIALS AND METHODS

Preliminary Investigation of the Infertile Couple

After the research studies demonstrating the AR to ionophore challenge test to be predictive of the fertilizing potential of a semen sample (5), the test was introduced for the routine assessment of sperm quality at PIVET Medical Centre. However, where satisfactory fertilization rates had already been demonstrated at previous IVF treatment cycles (i.e., $\geq 50\%$ fertilization rate where more than 4 oocytes were recovered), AR to ionophore challenge tests were considered unnecessary and not performed. During the work-up for infertility treatment, all couples completed a standardized investigatory profile (10). For the female, an assessment cycle evaluation was carried out in which follicular development and ovulation were assessed by hormonal and ultrasound (US) parameters. In addition, the female genital tract was evaluated by combined hysteroscopic and laparoscopic procedures. For the male, a semen analysis and an AR to ionophore challenge test were performed except where considered unnecessary from previous IVF experience. Semen analysis was carried out according to the World Health Organization (WHO) guidelines (11), and a sample was considered to have normal counts if it contained 20×10^6 or more total sperm per milliliter with a 50% motility.

Historically, PIVET has been reporting on treatments for severe male factor infertility over 10 years and, particularly with the introduction of pentoxifylline and micromanipulation, the clinic has attracted a large proportion of male factor patients. It is our experience that approximately 40% of patients have poor fertilization unless sperm stimulants or micromanipulation procedures are used. Therefore, the prevalence of poor fertilization obtained in the statistical analysis reflects the true prevalence of this problem among the patients in the clinic's IVF program.

Acrosome Reaction Test

In this study, the AR to ionophore challenge test was carried out as previously reported (5). In brief, a motile sperm fraction was prepared from semen samples by subjecting them to either overlay or sedimentation techniques (12). The motile sperm preparation was then divided into two aliquots; one was taken as the control and the other was exposed to 10 mmol/L calcium ionophore A23187 (Sigma Chemical Company, St Louis, MO) for 1 hour at 37°C. After exposure to calcium ionophore, the

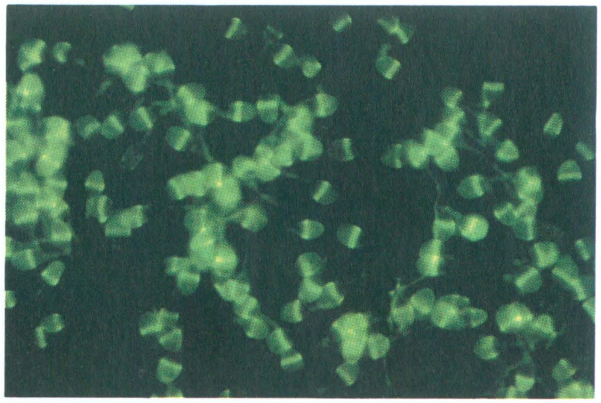

Figure 1 Spontaneous and ionophore-induced ARs in an FITC-conjugated PSA lectin-stained sperm preparation.

incubated sample (control + ionophore challenged) was centrifuged through a 60% Percoll density gradient medium (Pharmacia LKB Biotechnology AB, Uppsala, Sweden), and the sperm pellets resuspended in 95% ethanol. Ethanol-fixed sperm were transferred onto clean glass slides and stained with fluorescein isothiocyanate (FITC)-conjugated *Pisum sativum* lectin (PSA: catalog number L 0770, Sigma Chemical Company). The smear was then mounted under phosphate-buffered glycerol (1:9; pH 7.4) and examined under epifluorescence microscopy using the oil immersion objective (Fig. 1). When the AR to ionophore challenge reading was <10%, a second AR to ionophore challenge test was performed in which the motile sperm fraction was exposed to 1.0 mg/mL (3.6 mM) pentoxifylline for 30 minutes at 37°C (12) before ionophore challenge. If both results were reduced, a further test was performed with pentoxifylline combined with 0.87 mg/mL (3.0 mM) 2-deoxyadenosine.

Five main staining patterns were identified (Fig. 2): [I] complete staining of the acrosome, indicating acrosome intact sperm; [II] partial or patchy staining of the apical segment of the acrosome, indicating partially reacted sperm; [III] staining of the equatorial segment only, indicating completely reacted sperm; [IV] faint or ghost staining of the entire sperm head, indicating no acrosomal matrix; and [V] other irregular pattern or abnormally formed acrosome, indicating morphologically abnormal sperm. The proportion of sperm demonstrating a complete AR was expressed as the number of sperm counted of pattern III expressed as a percentage of I + II + III and calculated for both control and ionophore challenged suspensions. The difference between the two (ionophore less spontaneous) was considered to be the percentage of sperm in the population capable of responding to ionophore and referred to as the AR to ionophore challenge score. Patterns IV and V were excluded from the scoring and are thought to comprise most of the morphologically abnormal and dead sperm.

The AR to ionophore challenge results were maintained in the laboratory files during the 6-month study period and were not made available to either the patient or the embryologist before the IVF attempt. The results were subsequently made available at case review in planning future treatment cycles outside the study.

Assisted Reproduction Cycle

After the preliminary investigations of the couple, the mode of treatment for infertility was planned depending on the underlying factors revealed. It ranged from specific surgical procedures and noninvasive treatments to assisted reproductive techniques including IUI as well as procedures entailing oocyte recovery. For this study only, those patients were included who were on assisted reproductive treatment procedures involving IVF of all oocytes, that is, IVF-ET, tubal embryo stage transfer (TET), and pronuclear stage transfer (PROST); or supernumerary oocytes after GIFT. The study analyzed all cases (n = 121) treated on the first occasion between January and July 1992, that is, each couple is treated once only within the study. Donor sperm and micromanipulation techniques were used for patients who showed repeat

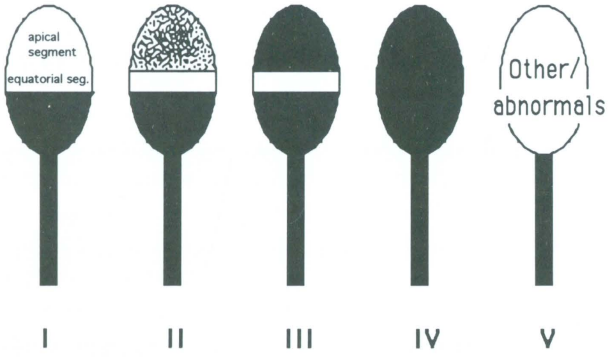

Figure 2 Schematic description of staining patterns of the sperm head with FITC-PSA. I, Complete staining. II, Partial or patchy staining. III, Staining of the equatorial segment only. IV, Faint or ghost staining of the entire sperm head. V, Morphologically abnormal and other patterns. The AR to ionophore challenge score is calculated as the ratio of pattern III over the total numbers in patterns I + II + III. It defines those sperm that have undergone a complete AR after ionophore challenge, that is, spontaneous reactions are deducted from the total reacted sperms.

fertilization failure in previous IVF attempts, and these cases were excluded from the study. Repeat treatments were undertaken on some couples during the study period but were excluded as the previous experience invariably influenced their management. Also, cases were excluded from the study if fewer than four preovulatory oocytes of good grading were retrieved.

Follicular development was achieved by GnRH analogue (Lucrin, Leuprorelin acetate; Abbott Australasia Pty. Ltd., Kurnell, New South Wales, Australia) used on a pituitary down regulation or "flare" regimen in combination with hMG (Pergonal; Serono, Aubonne, Switzerland) and FSH (Metrodin, Serono). Ovulation was induced with 10,000 IU hCG (Pregnyl; Organon Australia Pty. Ltd., Lane Cove, New South Wales, Australia), and the oocytes were collected 36 hours after hCG trigger. A transvaginal US-directed approach was used exclusively for the retrieval of oocytes. The oocytes were then graded before allocation (10), and special consideration was given to their nuclear maturity (13). To facilitate accurate grading, the egg mass was aspirated into a pipette and placed on a tilted Petri dish. This allowed the egg mass to spread so that oocyte details could be clearly identified. Oocytes with one polar body were considered metaphase II, no polar body as metaphase I, and nonvisible oocytes because of dense cumulus corona cells as immature or germinal vesicle. The oocytes were then randomly placed into culture tubes by an independent embryologist who was unaware of the patients' AR to ionophore challenge status.

A second embryologist prepared the semen samples using either the overlay or sedimentation techniques, and some motile fractions were treated with 3.6 mM pentoxifylline or pentoxifylline plus 3.0 mM 2-deoxyadenosine. The decision to use 2-deoxyadenosine was based primarily on the patients' requests, which were dependent on their past failed fertilization with pentoxifylline-treated sperm. Four to 6 hours after oocyte recovery, oocytes were inseminated randomly (using 50,000 to 100,000 sperm) with either control sperm or sperm treated with the stimulants. Oocytes were randomly selected for control or treated sperm insemination and mostly apportioned as $\frac{1}{2}$ to $\frac{1}{2}$. However, depending on semen characteristics, previous experience, and patient wishes, the proportion allocated to treated sperm ranged from $\frac{1}{3}$ to $\frac{2}{3}$, with pentoxifylline-treated sperm being favored for the poorer samples. Medicult IVF Medium (Medicult, Hvidovre, A/S, Denmark) was used for IVF, embryo culture, and sperm preparation. Fertilization was confirmed 18 to 20 hours after insemination, and a maximum of three pronuclear stage oocytes were transferred into the fallopian tube (PROST) or cleaving embryos (2- to 4-cell stage) were transferred either into the fallopian tube (TET) or uterine cavity (IVF-ET). For GIFT transfers, a maximum of three oocytes were transferred (best grades selected) together with 100,000 sperm prepared before the procedure. The remaining oocytes were then inseminated, fertilization confirmed, and cryopreserved if requested by the infertile couple. The patients were placed on luteal support therapy consisting of P 25 mg or 50 mg IM for 16 days from the day of oocyte recovery with 1,000 IU hCG on days 4, 7, and 10 unless the peak E_2 was greater than 12,000 pmol/L. In the latter group, no hCG was administered. Pregnancy was diagnosed by a serum β-hCG level of >25 IU/L on day 16 after ET and confirmed by transvaginal US 3 weeks later (week 7).

Ethics

PIVET Medical Centre has national accreditation by the Reproductive Technology Accreditation Committee and State Licencing under the Human Reproductive Technology Act, 1991 (Western Australia). PIVET's Institutional Ethics Committee is structured according to National Health and Medical Research Council guidelines that embrace the principles of the Helsinki Declaration of 1975, as revised in 1983. The protocols for sperm stimulation with pentoxifylline and 2-deoxyadenosine, along with patient information sheets and consent forms, are approved by the Ethics Committee.

Statistical Analysis

The semen analyses between different AR to ionophore challenge groups were compared using treatment factor one-way analysis of variance. The χ^2 statistic was applied for other comparisons involving fertilization rate, pregnancy rate, and incidence of failed fertilization among different AR to ionophore challenge groups.

RESULTS

Median oocyte recovery was 12 oocytes per collection, and a total of 1,418 oocytes were available for the study. The data obtained for IVF and pregnancy outcomes were correlated with the preliminary semen analysis and AR to ionophore challenge

scores (Table 1). The majority of semen samples in all categories of patients grouped according to the AR to ionophore challenge scores had normospermic counts. However, the mean counts were lower when the AR to ionophore challenge was reduced ($P < 0.05$) and lowest in the group in which the AR to ionophore challenge failed to normalize with pentoxifylline ($P < 0.01$) when compared with "AR to ionophore challenge not done" and "AR to ionophore challenge ≥ 10%" groups (Table 1). It is of interest that AR to ionophore challenge scores that remained low after pentoxifylline also failed to normalize with 2-deoxyadenosine, although occasional improvements were noted.

The IVF data for the respective AR to ionophore challenge groups are presented graphically for clarity (Fig. 3). The two groups with no AR to ionophore challenge done and an AR to ionophore challenge score of ≥10% gave equally satisfactory fertilization rates with control as well as sperm treated with sperm stimulants. Although the groups showed no overall differences, individual cases sometimes showed a marked reduction in fertilization (<50% oocytes) using pentoxifylline.

The fertilization rate for samples with poor AR to ionophore challenge tests (<10%) improved significantly with pentoxifylline treatment (147/261, 56.3%) when compared with control sperm (54/134, 40.3%; $P < 0.02$). Furthermore, a highly significant increase in fertilization rate was observed for samples where an improvement in the AR to ionophore challenge result with pentoxifylline was shown in the preliminary investigations (30/77, 39% compared to 68/120, 56.7%; $P < 0.005$). Samples that showed a poor AR to ionophore challenge despite the pentoxifylline treatment sometimes showed improvement in fertilization rates with pentoxifylline or pentoxifylline combined with 2-deoxyadenosine in comparison with the control sperm in the assisted reproduction attempt. This difference did not attain a statistically significant level. However, if the comparative ratio in terms of oocyte numbers persists, a significant level might be attained.

The predictive value of the AR to ionophore challenge test in a clinical setting for diagnosing patients with poor fertilization (<40% taken as poor fertilization) was assessed using various AR to ionophore challenge cutoffs, that is, 5%, 7.5%, and 10%. We selected 10% as the AR to ionophore challenge cutoff because of the higher sensitivity asso-

Table 1 Relationship of Preliminary Semen Analyses and AR to Ionophore Challenge Results to Fertilization Rate and Pregnancy Outcome

	Preliminary assessment				IVF rate			
		Semen analysis						
AR to ionophore challenge test	No. of patients	Motile count	Progressive count	Activity + to +++	Control	Pentoxifylline	Pentoxifylline + 2-deoxyadenosine	No. of pregnancies
		$\times 10^6$ mL				%		
AR to ionophore challenge not done	16	62.2 ± 9.7*	45.1 ± 8.4	++	66.1 (121/183)	60.9 (14/23)	—	4
AR to ionophore challenge ≥ 10%	30	61.1 ± 8.0	43.1 ± 5.9	++	67.6 (140/270)	70.5 (67/95)	66.7 (4/6)	6
AR to ionophore challenge <10%	41	39.2 ± 4.9†	29.8 ± 4.5	++	40.3 (54/134)	56.3 (147/261)‡	46.3 (37/80)	7
Pentoxifylline-AR to ionophore challenge ≥ 10% (control = 4.4% after pentoxifylline = 19.0%)	19	42.2 ± 8.0	33.5 ± 7.1	++	39.0 (30/77)	56.7 (68/120)§	40.0 (6/15)	3
Pentoxifylline-AR to ionophore challenge <10% (control = 3.1% after pentoxifylline = 4.1%)	15	26.2 ± 5.5‖	17.8 ± 4.3†‖	++	34.5 (10/29)	51.5 (51/99)	53.8 (14/26)	2

* Values are means ± SE.

† $P < 0.05$; motile count of AR to ionophore challenge <10% versus AR to ionophore challenge ≥10%; motile count of AR to ionophore challenge <10% versus AR to ionophore challenge not done; progressive count of pentoxifylline-AR to ionophore challenge <10% versus AR to ionophore challenge not done.

‡ $P < 0.02$, Fertilization rate of Pentoxifylline versus Control.

§ $P < 0.005$, Fertilization rate of Pentoxifylline versus Control.

‖ $P < 0.01$; motile count of pentoxifylline-AR to ionophore challenge <10% versus AR to ionophore challenge not done; motile count of pentoxifylline-AR to ionophore challenge <10% versus AR to ionophore challenge ≥10%; progressive count of pentoxifylline-AR to ionophore challenge <10% versus AR to ionophore challenge ≥10%.

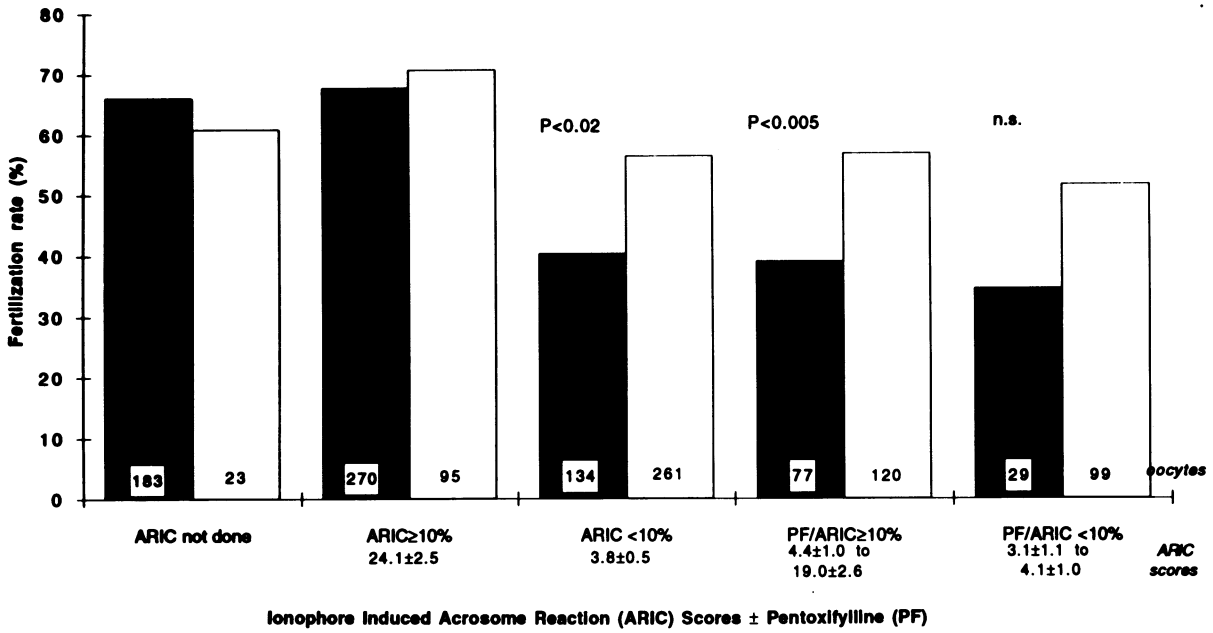

Figure 3 Fertilization rates with untreated sperm (■) and pentoxifylline-treated sperm (□) for patients with different AR to ionophore challenge scores.

ciated with highest negative predictive value and lowest false-negative rates at this level. The predictive values calculated in this study for the AR to ionophore challenge cutoff of 10% showed a sensitivity of 82.1% (23/28) with specificity of 56.1% (23/41). The positive predictive value of the test was 56.1% (23/41) and the negative predictive value 82.1% (23/28). The false-positive and false-negative rates were 43.9% (18/41) and 17.9% (5/28), respectively. The prevalence of poor fertilization in the patient population was 40.6% (28/69).

The rate of failed fertilization for each AR to ionophore challenge group was calculated using the cycles in which oocytes were randomly allocated for control and pentoxifylline-treated groups. For example, in the AR to ionophore challenge not done group, all 16 patients had oocytes inseminated with control sperm and only 5 patients had oocytes inseminated with pentoxifylline-treated sperm. None of the patients in this group had total fertilization failure. The data for the incidence of failed fertilization for all five AR to ionophore challenge groups are presented in Figure 4. The risk of failed fertilization was significantly reduced overall when pentoxifylline was used (Fig. 4). This was particularly noted in those cases in whom the AR to ionophore challenge score was <10% ($P < 0.01$) and appeared most marked when the AR to ionophore challenge score improved with pentoxifylline (pentoxifylline-AR to ionophore challenge).

DISCUSSION

This prospective, double-blind, and randomized controlled study shows that the AR to ionophore challenge test is valuable for predicting the potential capacity of a particular sperm sample to fertilize oocytes. Furthermore, the use of pentoxifylline in association with the AR to ionophore challenge test in the preliminary investigations identified more precisely those sperm samples that would benefit from the addition of sperm stimulants in the IVF attempt.

Other tests such as zona-free hamster assay (3), sperm-zona pellucida binding tests (14), hemizona assay (15), creatine kinase (16), and sperm morphology assessment using strict criteria (1) seem to predict the fertilization outcome in human IVF. However, the AR to ionophore challenge test described in this paper is a simple, inexpensive laboratory test that can be performed in any andrology laboratory with no restrictions associated with the availability of expertise or the testing material such as hamsters or human zonae. In the present study, the AR to ionophore challenge test with a 10% cutoff for delineating the subfertile or poor fertilization group gave a high sensitivity with a 44% false-posi-

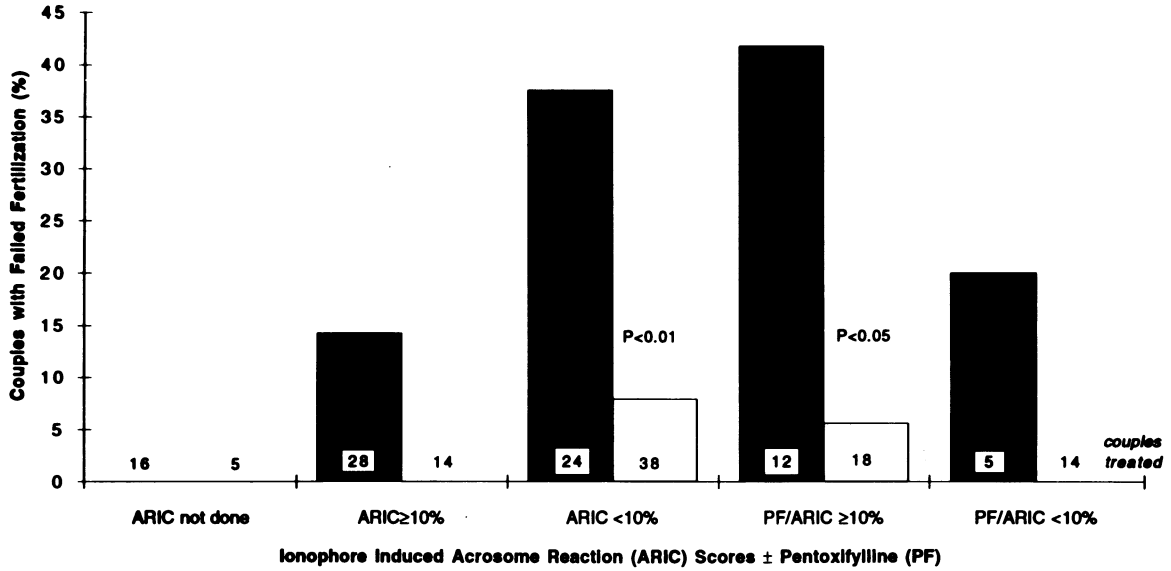

Figure 4 Couples with failed fertilization in the five patient groups categorized according to the AR to ionophore challenge scores after the use of untreated sperm (■), compared with pentoxifylline-treated sperm (□). No bar outline indicates no failed fertilization (0%).

tive rate. The high sensitivity of the test observed is different from our previous publication (54.1%) probably because of the different patient groups involved. It is likely that some patients have their oocytes inseminated with sperm treated with pentoxifylline even though the fertilization with control sperm is satisfactory. The high-negative predictive value with a corresponding low false-negative rate indicates that an AR to ionophore challenge score of ≥10% is highly predictive of good fertilization and that these patients can be treated safely without pentoxifylline in the sperm preparation. The patient group involved in this study was an unselected group in whom 40% would have had poor fertilization if pentoxifylline or micromanipulation procedures were not used. The 40.6% prevalence of failed or poor fertilization observed reflects the large number of male factor patients treated in this clinic.

Others have studied the AR with fluorescent staining employing different lectin binding sites and have suggested that precision may be improved by definitively excluding dead sperm, that is, by the hyperosmolar swelling test or vital staining. Mortimer et al. (17) have employed the Hoechst 33258 stain in combination with a fluorescent peanut agglutinin lectin that binds to the inner acrosomal membrane. The AR to ionophore challenge test stains the acrosomal matrix and scores the morphologically normal sperm within the highly motile fraction obtained from the sperm preparation. We have prepared a further study to determine if the additional cost and effort will improve the precision of the AR to ionophore challenge test but are more interested at this stage in encouraging the development of an automated reading system because the main limitation of the test relates to technician discomfort. The test is inexpensive but involves reading the stained slides within a dark room using epifluorescence microscopy. Some technicians may experience a nausea sensation akin to motion sickness, and the hardiest will score up to a maximum of 12 cases (reading up to 96 slides) over a period of 4 to 5 hours. Most require relief after 5 or 6 cases.

There appear to be several actions of pentoxifylline on human sperm, possibly acting through a common pathway resulting from the inhibition of phosphodiesterase and the resulting accumulation of cyclic adenosine monophosphate (cAMP) (18). There is an effect on several sperm motility parameters (19, 20), an enhancement of the AR in subfertile men (5, 21), and protection of the sperm plasma membrane through an antioxidant action (22, 23). This latter mechanism may be the most important because the majority of oligospermic samples that fail to fertilize oocytes exhibit elevated levels of reactive oxygen species (24). The excessive levels of reactive oxygen species may cause peroxidation of unsaturated fatty acids in the sperm plasma membrane. The damaged plasma membrane then loses its responsiveness to the calcium influx signal that triggers the AR. Pentoxifylline acts as an antioxi-

dant by removing peroxides formed from free radicals (superoxide anion and hydroxyl radical) possibly through an indirect manner. The high levels of cAMP probably reduce endoperoxide formation by inhibition of cyclo-oxygenase within the arachidonic acid pathway. 2-Deoxyadenosine also has an effect of increasing cAMP levels by an enhancement action on adenylate cyclase. However, no significant clinical benefit was noted by combining 2-deoxyadenosine with pentoxifylline in this study, although individual cases sometimes showed a benefit over pentoxifylline alone.

In vitro fertilization-embryo transfer was introduced as a potential treatment mode to overcome insoluble female factor infertility. However, it has long been considered that the methods could also be used for male factor infertility. The early approaches in this respect have relied on a trial-and-see approach with often unsatisfactory clinical outcomes when the result is total fertilization failure. In the present study, even though the average motile sperm counts for poor AR to ionophore challenge groups (AR to ionophore challenge $< 10\%$ and pentoxifylline-AR to ionophore challenge $< 10\%$) were low compared with other groups (AR to ionophore challenge not done and AR to ionophore challenge $\geq 10\%$), the majority of patients showed normal counts according to WHO criteria (11). With the guidance of the AR to ionophore challenge test, it is now possible to identify defective sperm samples even among those that are normospermic on semen analysis. The AR to ionophore challenge $\pm$ pentoxifylline laboratory test will delineate the majority of those cases that will benefit from sperm stimulation and minimize the risk of failed fertilization. In the "pentoxifylline-AR to ionophore challenge $\geq 10\%$" group, the employment of the AR to ionophore challenge test showed a high correlation between the improvement of AR to ionophore challenge scores and the improvement in fertilization rate for pentoxifylline-treated sperm. When there was no improvement in AR to ionophore challenge scores (pentoxifylline-AR to ionophore challenge $\leq 10\%$ group), only some improvement in fertilization rate was observed. This was probably due to comparatively low numbers of oocytes used. The apparent improvement in fertilization rate seen in this group may be due to other beneficial effects of pentoxifylline as described above. However, according to the data presented in this study, only the pentoxifylline-AR to ionophore challenge $\geq 10\%$ group showed a definite improvement in fertilization rate with pentoxifylline. Poor prognosis cases then will need to consider alternative options such as donor sperm treatments or micromanipulation to enhance fertilization. However, it is unknown if those cases not able to achieve fertilization with sperm stimulants will do so by a microinsemination method. From the available meager data, the technique of intracytoplasmic sperm injection appears the most relevant to explore because fertilization may be independent of the conventional AR (25).

In summary, the AR to ionophore challenge test is a reliable and inexpensive laboratory test that provides a useful guide in identifying the possible male factor patients in assisted reproduction. Furthermore, this test can be used as a tool in determining the majority of the samples that will benefit from pentoxifylline enhancement in the sperm preparation.

Acknowledgments. The authors thank the PIVET embryology and semenology staff, especially Anita Benbow and Sharon Apted for their help in carrying out the AR to ionophore challenge tests. We also thank Nick Costa, B.Ag.Sc., Ph.D., Murdoch University, Perth, Western Australia, for his advice on the statistical analysis.

REFERENCES

1. Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Oehninger S. Predictive value of abnormal sperm morphology in in vitro fertilization. Fertil Steril 1988;49:112–7.
2. Holt WV, Moore HDM, Hillier SG. Computer-assisted measurement of sperm swimming speed in human semen: correlation of results with in vitro fertilization assays. Fertil Steril 1985;44:112–9.
3. Albertsen PC, Chang TSK, Vindivich D, Robinson JC, Smyth JW. A critical method of evaluating tests for male infertility. J Urol 1983;130:467–75.
4. Bronson RA, Rogers BJ. Pitfalls of the zona-free hamster egg penetration test: protein source as a major variable. Fertil Steril 1988;50:851–4.
5. Cummins JM, Pember SM, Jequier AM, Yovich JL, Hartman PE. A test of the human sperm acrosome reaction following ionophore challenge (ARIC): relationship to fertility and other seminal parameters. J Androl 1991;12:98–103.
6. Talbot P, Chacon R. A triple-stain technique for evaluating normal acrosome reactions in human sperm. J Exp Zool 1981;215:201–8.
7. Cross NL, Morales P, Overstreet JW, Hanson FW. Two simple methods for detecting acrosome-reacted human sperm. Gamete Res 1986;15:213–26.
8. Mortimer D, Curtis EF, Miller RG. Specific labelling by peanut agglutinin of the outer acrosomal membrane of the human spermatozoon. J Reprod Fertil 1987;81:127–35.
9. Fénichel P, Hsi BL, Farahifar D, Donzeau M, Barrier-Delpech D, Yeh CJG. Evaluation of the human sperm acrosome reaction using a monoclonal antibody, GB24, and flourescence-activated cell sorter. J Reprod Fertil 1989;87:699–706.

10. Yovich JL, Grudzinskas JG. The management of infertility: a practical guide to gamete handling procedures. London: Heinemann, 1990.
11. World Health Organization. WHO Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 2nd ed. Cambridge: The Press Syndicate of the University of Cambridge, 1987: 27–8.
12. Yovich JM, Edirisinghe WR, Cummins JM, Yovich JL. Influence of pentoxifylline in severe male factor infertility. Fertil Steril 1990;53:715–22.
13. Mahadevan MM, Fleetham J. Relationship of a human oocyte scoring system to oocyte maturity and fertilizing capacity. Int J Fertil 1990;35:240–4.
14. Liu DY, Lopata A, Johnston WIH, Baker HWG. A human sperm-zona pellucida binding test using oocytes that failed to fertilize in vitro. Fertil Steril 1988;50:782–8.
15. Oehninger S, Toner J, Muasher SJ, Coddington C, Acosta AA, Hodgen GD. Prediction of fertilization in vitro with human gametes—is there a litmus test? Am J Obstet Gynecol 1992;167:1760–7.
16. Huszar G, Vigue L, Morshedi M. Sperm creatine phosphokinase M-isoform ratios and fertilizing potential of men: a blinded study of 84 couples treated with in vitro fertilization. Fertil Steril 1992;57:882–8.
17. Mortimer D, Curtis EF, Camenzind AR. Combined use of fluorescent peanut agglutinin lectin and Hoechst 33258 to monitor the acrosomal status and vitality of human spermatozoa. Hum Reprod 1990;5:99–103.
18. Tash JS, Means AR. Cyclic adenosine 3′,5′ monophosphate, calcium and protein phosphorylation in flagellar motility. Biol Reprod 1983;28:75–104.
19. Rees JM, Ford WCL, Hull MGR. Effect of caffeine and of pentoxifylline on the motility and metabolism of human spermatozoa. J Reprod Fertil 1990;90:147–56.
20. Tesarik J, Thébault A, Testart J. Effect of pentoxifylline on sperm movement characteristics in normozoospermic and asthenozoospermic specimens. Hum Reprod 1992;7:1257–63.
21. Tesarik J, Mendoza C. Sperm treatment with pentoxifylline improves the fertilizing ability in patients with acrosome reaction insufficiency. Fertil Steril 1993;60:141–8.
22. Aitken RJ, West KM. Analysis of the relationship between reactive oxygen species production and leucocyte infiltration in fractions of human semen separated on Percoll gradients. Int J Androl 1990;13:433–51.
23. Gavella M, Lipovac V, Marotti T. Effect of pentoxifylline on superoxide anion production by human sperm. Int J Androl 1991;14:320–7.
24. Aitken RJ, Clarkson JS, Hargreave TB, Irvine DS, Wu FC. Analysis of the relationship between defective sperm function and the generation of reactive oxygen species in cases of oligozoospermia. J Androl 1989;10:214–20.
25. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992;340:17–9.