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**A Stimulated Acrosome Reaction Test
as a Prognostic Factor in In Vitro Fertilization**

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= Abstract

It is well known that the clinical test for responsibility of accurate fertilization capacity in male partners is very important to diagnose and treat the infertility. However, it has been reported that the traditional semen analysis cannot accurately predict fertilization and pregnancy potential.

The present study was performed to evaluate the acrosomal reaction to ionophore challenge (ARIC) test as a prognostic indicator for fertilization of sperm and oocyte in an in vitro fertilization and embryo transfer (IVF-ET) program. From March 1996 to February 1997, 30 couples undergoing IVF program were allocated to this study group. All female partners in the study group were 35 years old or less and their serum level of basal follicle stimulating hormone (FSH) and estradiol (E_2) were normal. All the male partners have normal parameters of semen analysis. The ARIC tests were performed on the day of ovum pick up and in vitro insemination in all the male partners. The controlled ovarian hyperstimulation (COH) using luteal long protocol of gonadotropin releasing hormone (GnRH) agonist was used in all couples for IVF-ET.

The acrosomal reaction with 10 μ l of 10% DMSO was induced spontaneously in $10.1 \pm 9.8\%$, and acrosomal reaction with calcium ionophore A 23187 was induced in $27.4 \pm 18.1\%$, and the ARIC value was $17.4 \pm 16.2\%$. There were no significant correlation between the ARIC value and the fertilization rate ($r^2 = 0.044$, $p = 0.268$). There were also no significant correlation between the ARIC value and the percentage of the grade I, II embryos ($r^2 = 0.046$, $p = 0.261$).

On the basis of above results, it was suggested that ARIC test might not be a useful prognostic indicator for fertilization in IVF-ET in male partners with normal parameters of conventional semen analysis. We guessed that IVF-ET could be performed to the patients primarily without universal application of ARIC test to all male partners, and if fertilization failure occurs, the microassisted fertilization (MAF) such as intracytoplasmic sperm injection (ICSI) might be used as an alternative mode of treatment with acceptable success rate.

Key words : Acrosomal reaction to ionophore challenge (ARIC) test, Fertilization capacity, Intracytoplasmic sperm injection (ICSI)

(semen analysis)가
(motility) (morphology)
가 (fertilization capacity)
(Cockett et al., 1975).

(computer-aided semen analysis, CASA), (hamster sperm penetration
assay, SPA), (hypoosmotic swelling test, HOST),
(in vitro test of sperm penetration into mucus),
(sperm morphology evaluation using strict criteria, SMEUSC)

SPA SMEUSC가 가 SPA
가 , , ,
, SMEUSC 가 ,
, 가 ,
SPA SMEUSC

(capititation) (acrosomal
reaction) (Bedford, 1983).

가
1991 Cummins
가
(Fenichel et al, 1991; Pampiglione et al., 1993;
Henkel et al., 1993)

(in vitro fertilization and embryo transfer, IVF-ET)
calcium ionophore FITC-PSA
가

(acrosomal reaction to ionophore

challenge test, ARIC test)

.

1.

1996 3 1997 2

35 , 3
(follicle stimulating hormone, FSH) (estradiol, E₂) 가
가 (Wood et al.,
1985; Molloy et al., 1987; Muasher et al., 1988; Scott et al., 1989; Licciardi et al., 1991)

2.

2-3
120 ml polypropylene
30-60

1)

(World Health Organization, WHO, 1992)

10 μ l Makler counting chamber(Sefi Medical)
(%)

2)

5 ml Ham's F-10(+0.3% HSA) 600 g 5
 3 ml Ham's F-10(+0.3% HSA) 600 g 10
 Ham's F-10(+1.0% HSA) 가 1 37 ° C, 5% CO₂
 가
 10 µl 10%
 DMSO(dimethyl sulfoxide) 37 ° C 1
 100 mmol/l, 10 µl calcium ionophore A 23187(Sigma,
 U.S.A.) 5 mM DMSO 가 Calcium ionophore
 10, 20, 30 mM F10 가
 300 µl 80% Percoll 가 600 g 15
 30 4 ° C 50 µl, 95% ethanol
 ethanol , 50
 µg/ml FITC(fluorescein isothiocyanate)-conjugated pisum sativum lectin(PSL, L 0770, Sigma,
 U.S.A.) 4 ° C
 15
 5가 A 가 가
 , B 가
 , C , D
 가 E
 C /A, B, C
 x 100(%) D E

3)

GnRH-a(Decapeptyl, D-Trp-6-LH, Ferring, Malmo, Sweden)

(luteal phase long protocol) (Neveu et al., 1987; Tan et al., 1992 & 1994). 가 14

21 GnRH-a 0.1 mg

GnRH-a 3-5 (Leopard, B & K, Denmark) 10mm

GnRH-a 가

human menopausal gonadotropin(hMG; Humegon, Organon, Holland) human follicle stimulating hormone(hFSH; Metrodin, Serono, Switzerland)

(step-down fashion) , hMG hFSH

4 ,

(leading follicle) 18 mm

16 mm 가 3 human chorionic gonadotropin(hCG; Pregnyl, Organon, Holland) 10,000 IU

E₂ 가 estradiol MAIA kit(CIBA-Corning Diagnostics, U.S.A) (radioimmunoassay, RIA)

intraassay variance interassay variance 7% 10%

18-20 48 가

Jones (1983) (transfer catheter)

5 ,

progesterone in oil(Progest, Samil Phar., Korea) 50mg

(luteal support)

11-12 β-hCG 3 mIU/ml

1 (gestational sac) 가

(clinical pregnancy)

β-hCG hCG MAIACLONE kit(Serono Diagnostics, MA) IRMA가

interassay variance intraassay variance 10% 5%

4)

ARIC value
analysis

1, 2
 $p < 0.05$

regression
.

1996 3 1997 2 30
 1 20%
 32.8 ± 4.1 35.9 ± 4.7
 68.7 ± 55.4
 가 19 , 가 3 , I, II 8
 3.3 ± 1.3 ml, $108.2 \pm 109.2 \times 10^6$ /ml,
 $73.9 \pm 20.3\%$, $56.2 \pm 21.4\%$

(Table 1).

75IU 36.5 ± 11.5 9.7 ± 1.5
 9.0 ± 4.4
 7.6 ± 4.6 $81.7 \pm 20.2\%$
 4.9 ± 1.3 (Table 2).

ARIC DMSO $10.1 \pm 9.8\%$
 , A23187 $27.4 \pm 18.1\%$
 ARIC value $17.4 \pm$
 16.2% (Table 2). ARIC value Fig. 2
 $(r^2 = 0.044, p = 0.268)$. ARIC value
 I, II (grade I, II) $(r^2 =$
 $0.047, p = 0.261, \text{Fig. 3})$. 1 ARIC value 5.1%

Table 1. Clinical characteristics of patients

No. of patients	30
Age of patients (yrs)	32.8 \pm 4.1
Age of husbands (yrs)	35.9 \pm 4.7
Duration of infertility (mos)	68.7 \pm 55.4
Patients with primary infertility	12/30 (40.0%)
Indications of IVF-ET	
Tubal factor	19
Endometriosis (Stage I, II)	3
Chronic anovulation	8

Values are means \pm SD.

Table 2. Results of IVF-ET and ARIC test of the study populations

No. of patients	30
No. of gonadotropin ampules (75IU)	36.5 ± 11.5
Duration of gonadotropin administration (days)	9.7 ± 1.5
No. of oocytes retrieved	9.0 ± 4.4
No. of oocytes fertilized	7.6 ± 4.6
Fertilization rate (%)	81.7 ± 20.2
No. of embryos transferred	4.9 ± 1.3
Pregnancy rate per patient	5/30 (40.0%)
Spontaneous acrosomal reaction	10.1 ± 9.8
Induced acrosomal reaction	27.4 ± 18.1
ARIC value	17.4 ± 16.2

Values are means ± SD.

Fig. 1. Staining patterns of the sperm head with FITC-PSA

Fig. 2. Correlation between fertilization rate and ARIC values of the patients

Fig. 3. Correlation between proportion of grade I, II embryos and ARIC values of the patients

40% , (microassisted fertilization, MAF) 가 WHO(1992) 가 (Cockett et al., 1975; Dunphy et al., 1989). 가 (azoospermia) 가 (Liu & Baker, 1992), 가 (Jequier & Ukome, 1983). 가 CASA, SPA, SMEUSC . CASA (culvilinear velocity, VCL) (amplitude of lateral head displacement, ALH) (Davis and Katz, 1993). Yanagimachi (1976) SPA 가 0% 16% (Margalioth et al., 1989), . SMEUSC 가 (Kruger et al., 1986; , 1994), Kruger (1986) 4.0%

, (, 1994) 2.5% .
 ARIC 가
 (Cummins et al., 1991; Fenichel et al., 1991; Pampiglione et al., 1993;
 Calvo et al., 1994; Yovich et al., 1994). (acrosome)
 (acrosomal membrane)
 (plasma membrane) (vesicle)
 acrosin, hyaluronidase, hydrolytic enzyme
 (zona pellucida)
 (cumulus cell)(Stock et al., 1989), (Cross et al., 1988),
 (follicular fluid)(Tesarik, 1985) , calcium ionophore(Tesarik,
 1985) .
 , 가 가
 Calvo (1994) , 가 가
 ,
 SMEUSC
 5% ,
 ARIC 가 , 2.5
 , ARIC ,
 , (quality control)가
 calcium ionophore .
 가 calcium ionophore Yovich (1994)
 ,
 10% ,
 Pampiglione (1993)
 31.3%
 . ARIC
 , ()
 () 가
 가 (Cummins et al., 1991; Fenichel et al., 1991).

1996 3 1997 2

ARIC 가

1. 30 ARIC 가 1

20%

2. 32.8 ± 4.1 35.9 ± 4.7

68.7 ± 55.4

가 19 , 가 3 , I, II 8

3. 3.3 ± 1.3 ml, 108.2 ± 109.2 x 10⁶/ml,

73.9 ± 20.3%, 56.2 ± 21.4%

4. 36.5 ± 11.5

9.7 ± 1.5 9.0 ± 4.4

7.6 ± 4.6 81.7

± 20.2% 4.9 ± 1.3

5. ARIC DMSO 10.1 ± 9.8 ,

A23187 27.4 ± 18.1

ARIC value 17.4 ± 16.2

6. ARIC value I, II

, ARIC

ARIC

가 ICSI

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