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# CLINICAL ASSISTED REPRODUCTION

## A Prospective, Randomized Comparison of Two Commercial Media for ICSI and Embryo Culture

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***Purpose:*** *The aim of this prospective, randomized study was to compare the results obtained in ICSI with two culture media, P-1 (Irvine Scientific) and IVF-50 (Scandinavian IVF Science).*
***Methods:*** *A total of 182 patients undergoing ICSI treatment were randomly included in this study and divided in two groups: Group I: P-1 medium (*n = *91) or Group II: IVF-50 medium (*n = *91). All the embryos were transferred on the second day.*
***Results:*** *Patient age did not differ (*p = *.29) between Group I (34.8 ± 4.8) and Group II (34.0 ± 4.5). The number of oocytes retrieved from Group I (10.6 ± 6.7) was also similar (*p = *.49) to that retrieved from Group II (11.1 ± 6.4). In addition, there was no difference (*p = *.25) in the number of oocytes retrieved at metaphase II between Group I (7.9 ± 4.6) and Group II (8.7 ± 4.6). Normal fertilization rates, abnormal fertilization rates, and cleavage rates were similar (*p = *.62,* p = *.48, and* p = *.9, respectively) between Group I (68.4 ± 23.3%, 6.7 ± 10.3%, and 98.7 ± 4.6%) and Group II (65.3 ± 26.2%, 9.0 ± 13.8%, and 98.9 ± 3.9%, respectively). The embryo score was also similar (*p = *.62) for both groups (Group I: 31.9 ± 14.0 and Group II: 33.4 ± 15.8). There was no difference in the number of embryos transferred (*p = *.69) between Group I (2.8 ± 1.0) and Group II (2.8 ± 1.1). In addition, pregnancy rates/puncture, pregnancy rates/transfer, implantation rates, and abortion rates were also similar for Group I (36.2%, 37.0%, 17.4%, and 12.1%, respectively) and Group II (31.8%, 33.7%, 15.8%, and 10.3%, respectively) (*p = *.64,* p = *.75,* p = *.72, and* p = *1.0, respectively).*
***Conclusions:*** *There were no differences in the results obtained with culture media P-1 (Irvine Scientific) and IVF-50 (Scandinavian IVF Science) for ICSI and embryo culture.*

**KEY WORDS:** culture medium; embryo implantation; ICSI.

### INTRODUCTION

In vitro development of human embryos for 48 to 72 h can be achieved using a wide variety of media, ranging from simple balanced salt solutions to more complex media (1).

The culture media used for therapeutic in vitro fertilization (IVF) may be prepared "in house" or commercially, when they are supplied ready to use and specified for use in IVF. The disadvantages of preparing media "in house" include batch-to-batch variation, the difficulty of establishing reliable quality control, and the labour-intensive nature of their preparation. For this reason, commercially available and ready-to-use, quality-controlled media are an attractive alternative for many IVF centres.

The aim of this study was to compare two different commercially available media used for ICSI: P-1 (Irvine Scientific) medium and IVF-50 (Scandinavian IVF Science) medium.

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## MATERIALS AND METHODS

A total of 182 patients submitted to ICSI were divided prospectively and at random into two groups of 91 patients: Group I, P1 (Irvine Scientific) medium and Group II, IVF-50 (Scandinavian IVF Science) medium. Patient participation in each group was random, by drawing lots, using a randomization table previously elaborated for the study. The P1 medium is a synthetic human tubal fluid (HTF) medium with the addition of the amino acid taurine, without glucose, protein, or phosphate, but containing 10 $\mu$g/mL gentamicin. The IVF-50 medium consists of a modified HTF medium with 25 mM bicarbonate, 10 mg/mL human serum albumin, and penicillin G.

For ovarian stimulation, the second phase was blocked with nafarelin acetate at the dose of 400 $\mu$g per day (Synarel, Searle). Habitually, 14 days after the use of the analogue, with establishment of the blockade (menstruation), the administration of recombinant FSH (Puregon, Organon) was started at a fixed dose of 150 or 300 IU for a period of 7 days (2). On the eighth day of ovarian stimulation, follicular development started to be monitored by vaginal ultrasound only, with the doses of pure FSH being adapted according to ovarian response. When a minimum of three follicles measuring >17 mm in diameter were observed, hCG was administered at the dose of 5,000–10,000 IU (3).

Oocytes were retrieved from the follicles by ultrasound-guided transvaginal puncture 34–36 h after hCG administration. After identification in follicular fluid, the oocytes were classified in terms of maturity and transferred to Group I in P1 medium containing 3% human serum albumin (HAS, Irvine Scientific) and to Group II in IVF-50 medium. The cumulus-corona complex was removed by exposure to a Type IV hyaluronidase solution (H-4272, Sigma, USA) at the concentration of 40 IU/mL and the denuded oocytes were incubated in P1 medium + 3% HAS (Group I) and IVF-50 medium (Group II) until the time for ICSI.

Discontinuous gradients—Isolate (Irvine Scientific) for Group I and Sperm-Prep-100TM (Scandinavian IVF Science AB, Sweden) for Group II—were used to separate the spermatozoa from seminal fluid in the 40% and 90% fractions.

ICSI was performed under an inverted Olympus IMT-2 microscope equipped with a Hoffman lens system and coupled to an automatic micromanipulator and injectors. A 10% polyvinylpyrrolidine solution (PVP, Irvine Scientific) for Group I and 10% polyvinylpyrrolidine solution (ICSI-100, Scandinavian IVF Science) for Group II were used to immobilize the spermatozoa. This viscous PVP solution is specially produced for the ICSI procedure and is previously tested for the presence of DNA and endotoxins (4). The oocytes were transferred in microdrops of modified HTF medium with Hepes (Irvine) under oil (Sigma) in Group I and in microdrops of modified HTF medium with HEPES (Gamete, Scandinavian IVF Science) under oil (Ovoil Scandinavian IVF Science) for Group II for the ICSI procedure.

The first polar corpuscle was positioned at 6:00 o'clock and a micropipette with the spermatozoon was introduced horizontally into the ooplasm at the 3:00 o'clock position. Gentle suction was carefully applied to break the oolemma and to visualize the presence of ooplasm inside the micropipette, confirming the intracytoplasmic deposition of the sperm. The immobilized sperm was inserted together with the aspirated ooplasm and with the smallest possible amount of PVP (4). After injection, the oocytes were cultured in P1 medium + 10% serum substitute supplement (SSS, Irvine Scientific) for Group I, and in IVF-50 medium for Group II. Fertilization was observed 16–19 h after the procedure to determine the presence or absence of pronuclei. A normal fertilization process was defined on the basis of the formation of two distinct pronuclei. The zygotes were then transferred to P1 medium + 20% SSS for Group I, and to IVF-50 medium for

**Table I.** Comparison of ICSI Outcome Using Two Different Culture Media

| | Group I (P-1) | Group II (IV-50) | *p* |
|---|---|---|---|
| No. of cycles | 91 | 91 | |
| Age (years) | 34.8 ± 4.8 | 34.0 ± 4.5 | .29[a] |
| Oocytes collected | 10.6 ± 6.7 | 11.1 ± 6.4 | .49[b] |
| Metaphase II oocytes | 7.9 ± 4.6 | 8.7 ± 4.6 | .25[b] |
| Normal fertilization rate (%) | 68.4 ± 23.3 | 65.3 ± 26.2 | .62[b] |
| Abnormal fertilization rate (%) | 6.7 ± 10.3 | 9.0 ± 13.8 | .48[b] |
| Cleavage rate (%) | 98.7 ± 4.6 | 98.9 ± 3.9 | .90[b] |
| Embryos transferred | 2.8 ± 1.0 | 2.8 ± 1.1 | .69[b] |
| Embryo score | 31.9 ± 14.0 | 33.4 ± 15.8 | .62[b] |
| Pregnancy rate/puncture (%) | 36.2 (33/91) | 31.8 (29/91) | .64[c] |
| Pregnancy/transfer (%) | 37.0 (33/89) | 33.7 (29/86) | .75[c] |
| Implantation rate (%) | 17.4 (44/252) | 15.8 (38/240) | .41[c] |
| Abortion rate (%) | 12.1 (4/33) | 10.3 (3/29) | 1.0[c] |

[a] Student's *t* test.
[b] Mann–Whitney test.
[c] Fisher's Exact test.

Group II. Embryo transfer (ET) was routinely performed after 48 h in culture, and supernumerary embryos were cryopreserved at the end of the second day.

On the day of ET the embryos were scored as follows: Grade 4—regular and symmetrical blastomeres with the absence of fragmentation; Grade 3—irregular blastomeres or less than 10% fragmentation; Grade 2—10 to 50% blastomere fragmentation; Grade I—more than 50% fragmentation, or embryo in the pronucleus stage. The morphological grade of the embryo was then multiplied by the number of blastomeres in order to obtain a qualitative score for each embryo. The scores of all embryos transferred per patient were summed to obtain the embryo score for each patient.

Data were analyzed statistically by the Student's *t* test, the Mann–Whitney test, and the Fisher exact test.

## RESULTS

Patient age did not differ ($p = .29$) between Group I ($34.8 \pm 4.8$) and Group II ($34.0 \pm 4.5$). The number of oocytes retrieved from Group I ($10.6 \pm 6.7$) was also similar ($p = .49$) to that retrieved from Group II ($11.1 \pm 6.4$). In addition, there was no difference ($p = .25$) in the number of oocytes retrieved at metaphase II between Group I ($7.9 \pm 4.6$) and Group II ($8.7 \pm 4.6$). Normal fertilization rates, abnormal fertilization rates, and cleavage rates were similar ($p = .62$, $p = .48$, and $p = .9$, respectively) for Group I ($68.4 \pm 23.3\%$, $6.7 \pm 10.3\%$, and $98.7 \pm 4.6\%$) and Group II ($65.3 \pm 26.2\%$, $9.0 \pm 13.8\%$, and $98.9 \pm 3.9\%$, respectively). The embryo score was also similar ($p = .62$) for both groups (Group I: $31.9 \pm 14.0$ and Group II: $33.4 \pm 15.8$). There was no difference in number of embryos transferred ($p = .69$) between Group I ($2.8 \pm 1.0$) and Group II ($2.8 \pm 1.1$). In addition, pregnancy rates/puncture, pregnancy rates/transfer, implantation rates, and abortion rates were also similar for Group I (36.2%, 37.0%, 17.4%, and 12.1%, respectively) and Group II (31.8%, 33.7%, 15.8%, and 10.3%, respectively) ($p = .64$, $p = .75$, $p = .72$, and $p = 1.0$, respectively).

## DISCUSSION

The use of commercially prepared media is becoming increasingly widespread in all aspects of assisted conception, with a number of products now available for procedures ranging from egg collection, embryo culture, and semen preparation, to semen and embryo cryopreservation. Although the increased use of commercial products has largely been due to practical and economic considerations, since the preparation of a number of different media "in house" can be very labour intensive and requires expensive equipment, there is also a powerful argument in favour of better quality control with mass production than is possible in small institutions (5).

Several studies comparing different commercial culture media for IVF are available in the literature. Among them is the study by Staessen *et al.* (6) who, in a first study from December 1993 to June 1994, compared Ménézo B2 medium to Medi-Cult (Medi-Cult Universal IVF medium). In this self-controlled study, sibling oocytes cultured in Ménézo B2 medium showed a normal fertilization rate similar to that for those cultured in Medi-Cult: ($62.9 \pm 33.3$)% and ($61.0 \pm 33.0$)% respectively, NS). In a second autocontrolled study conducted from November 1995 to March 1996, they compared Ménézo B2 medium to BM1 (Ellios Bio-Media) medium. The normal fertilization rate for the sibling oocytes was similar when they were cultured in Ménézo B2 and in BM1: $66.9 \pm 27.5\%$ versus $62.0 \pm 31.7\%$ (NS). After further culture of the fertilized oocytes, no difference was apparent in either study as regards the morphological characteristics of the embryos cultured in the different media. As regards the implantation potency of the embryos obtained in the different media, data were collected from nonrandomized transfers and the study indicated that the three commercial media with a different composition are equally able to sustain fertilization and embryo development until transfer.

Karamelegos and Bolton (5) in a prospective, randomized study compared the use of Earle's balanced salt solution (EBSS) prepared "in house" with that produced commercially (Medi-Cult) in 448 IVF cycles. Outcome was assessed in terms of fertilization and cleavage rates, embryo morphology, and implantation rates following embryo transfer. The only differences that were found between the two media in any outcome parameters were in the number of cycles with failed fertilization (1/218 for "in house" medium compared with 10/230 for commercially prepared medium; $p = .0186$) and the number of embryos per cycle that had cleaved to the 4-cell stage by 46–49 postinsemination was significantly higher in Medi-Cult medium than in EBSS medium ($p < .001$). The authors concluded that the pratical benefits of using an "off the shelf" product almost certainly

outweigh any disadvantages that may arise through a possible, but very slight increase in the rate of idiopathic failed fertilization.

Several studies have been conducted to compare different culture media for the ICSI procedure. In 1998, Parinaud *et al.* (7) tested a new culture medium (EllioStep2, Ellios Bio-Media, Paris, France) specially designed for the first cleavages and compared it with two conventional media, i.e., BM1 (EllioStep2, Ellios Bio-Media, Paris, France) and IVF-50 (Scandinavian IVF Science, Gothemburg, Sweden). A total of 416 ICSI attempts were randomly performed using one medium or the other. After sperm injection, oocytes were incubated in EllioStep2, BM1, or IVF-50. Embryo quality, pregnancy, and implantation rates, and number of frozen embryos were compared for the different media. The percentage of fair embryos (grades 4 and 3) was significantly higher when EllioStep2 was used than when oocytes were cultured in BM1 medium (545 versus 47%; $p < .01$) or in IVF-50 medium (69 versus 61%; $p < .01$). The pregnancy rate per transfer and the implantation rate were not significantly higher with EllioStep2 than with BM1 or IVF-50. However, the percentage of embryo freezings per attempt was significantly higher with EllioStep2 than with BM1 (47/105 versus 28/105, $p < .01$).

In another study, De Clerck *et al.* (8) compared the Sydney IVF culture system (commercialized by Cook IVF) with culture in Ménézo B2 medium routinely used at their centre. At ovum retrieval, the oocytes of each patient were alternately washed in Earle's medium and cultured in B2 medium, or washed in oocyte washing buffer and cultured in fertilization medium (Cook IVF). Eighty-four patients undergoing conventional IVF and 40 patients undergoing ICSI were included in the study. At the time of transfer, the best embryos were selected, irrespective of the culture system. The results of the study indicated that there was no significant difference between the two culture systems in terms of normal fertilization rate. In Cook IVF medium, embryo cleavage (morning of day 2) was retarded, but morphological quality was significantly better. More embryos from Cook IVF were used for transfer.

The P1 (Irvine Scientific, USA) and IVF-50 (Scandinavian IVF Science, Sweden) culture media are used by several assisted reproduction groups all over the world; however, we found no study comparing them for the ICSI procedure.

The two culture media, P1 and IVF-50, are modifications of the HTF medium, with the following differences: P1 uses gentamicin as an antibiotic and IVF-50 uses penicillin G, P1 contains no glucose and IVF-50 contains glucose. In addition, IVF-50 is supplemented with human serum albumin and P1 is protein free but contains the amino acid taurine.

In the present study we performed a prospective and randomized analysis of 182 patients divided into two groups. From oocytes retrieval to embryo transfer, only products of Irvine Scientific were used for Group I and only products of Scandinavian IVF Science were used for Group II, so that the evaluation of the culture system would be as real as possible.

Our results showed no significant differences between the two culture systems in terms of normal and abnormal fertilization rates, embryo cleavage rates, embryo quality evaluated by embryo score, pregnancy rate per oocyte retrieval and per embryo transfer, implantation rate, or abortion rate.

We conclude that both culture media used, P1 medium and IVF-50 medium, for the ICSI procedure and for later embryo culture with transfer on the second day are equally effective and can be used depending on the ease and availability of acquisition.

## REFERENCES


1. Bavister BD: Culture of preimplantation embryos: Facts and anti facts. Hum Reprod Update 1995;1:91–148
2. Franco JG Jr, Baruffi RLR, Coelho J, Mauri AL, Petersen CG, Garbellini E: A prospective and randomized study of ovarian stimulation for ICSI with recombinant FSH versus highly purified urinary FSH. Gynecol Endocrinol 2000;14:5–10
3. Oliveira JBA, Baruffi RLR, Mauri AL, Petersen CG, Borges MC, Franco JG Jr: Endometrial ultrasonography as a predictor of pregnancy in an in-vitro fertilization programme after ovarian stimulation and gonadotrophin-releasing hormone and gonadotrophins. Hum Reprod 1997;12:2515–2518
4. Svalander P, Forsberg AS, Jakobsson AH, Wikland M: Factors of importance for the establishment of a successful program of intracytoplasmic sperm injection treatment for male infertility. Fertil Steril 1995;63:828–837
5. Karamelegos C, Bolton VN: A prospective comparison of "in house" and commercially prepared Earle's balanced salt soltion in human in-vitro fertilization. Hum Reprod 1999;14: 1842–1846
6. Staessen C, Janssenwillen C, De Clerck E, Van Steirteghem A: Controlled comparison of commercial media for human in-vitro fertilization: Ménézo B2 medium versus Medi-Cult universal and BM1 medium. Hum Reprod 1998;13:2548–2554
7. Parinaud J, Veiga A, Vieitez G, Busquets A, Milhet P, Calderon G, Richoilley G, Barri P: Randomized comparison of three media used for embryo culture after intracytoplasmic sperm injection. Hum Reprod 1998, 13(Suppl 4):212–217
8. De Clerck E, Janssens R, Joris H, Verhein G, Van Steirteghem A: Prospective, controlled, randomized culture systems: Ménézo B2 medium and Cook IVF medium (Abstract Book). Hum Reprod 2000;15:34