Prospective Randomized Comparison of Two Embryo Culture Systems: P1 Medium by Irvine Scientific and the Cook IVF Medium

Dalit Ben-Yosef,^{1,3} Ami Amit,¹ Foad Azem,¹ Tamar Schwartz,¹ Tania Cohen,¹
Nava Mei-Raz,¹ Ariela Carmon,¹ Joseph B. Lessing,¹ Yuval Yaron²

Submitted November 14, 2003; accepted July 16, 2004

Purpose: To compare the efficacy of two commercially available in vitro fertilization (IVF) and embryo culture media systems: the glucose-free P1 Medium supplemented with 20% synthetic serum substitute (SSS) (Irvine Scientific), and the Cook IVF Medium (Cook, Australia).

Methods: A prospective randomized study. Medical center-based IVF Unit affiliated to the Faculty of Medicine of Tel Aviv University. IVF patients were randomly assigned to either P1 Medium supplemented with 20% SSS (182 patients, 196 cycles) or Cook Medium (167 patients, 179 cycles).

Results: Fertilization rates were similar with both media (52.3 ± 26.1 and 53.8 ± 27.6 , respectively). Likewise, no difference was found in morphological characteristics and grading of cultured embryos. However, a significantly higher proportion of the embryos incubated in the P1 Medium reached the four-cell stage on day 2 or the 6-cell stage on day 3 post-fertilization, compared to those incubated in Cook Medium (54.3% vs. 41.9%, $p < 0.0001$). Clinical pregnancy and delivery rates were improved when oocytes and embryos were cultured in P1 Medium. Finally, Implantation rate was significantly higher in the P1 Medium Group (9.9% vs. 6%, respectively).

Conclusions: Our results suggest that the P1 Medium may be associated with a higher embryo cleavage rate and improved implantation rates compared to the Cook IVF Medium.

KEY WORDS: IVF; culture media; preimplantation embryo development; implantation rate.

INTRODUCTION

The oviduct is the natural environment of the mature postovulatory human oocyte, where it is surrounded by the oviductal fluid. During the first mitotic divisions, the embryo passes along the oviduct to reach the uterus at about 4 days postfertilization. The in vivo environment of the preimplantation embryo changes along its route, as do its metabolic requirements.

Studies on the metabolism of the preimplantation cleaving embryo enabled the formulation of culture media capable of sustaining normal development (1). To satisfy these changing requirements, sequential culture media have been developed. These are now commercially available, ready-to-use, and quality-controlled. Using these commercial media, preimplantation embryos can routinely culture up to the blastocyst stage, and following embryo transfer (ET), result in normal offspring. While the benefit of using "off the shelf" products almost certainly outweighs any disadvantage (2), these new culture media are still under investigation and their optimal composition remains to be determined. Further modifications of these media may well result in

¹ The IVF Unit, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel.

² Prenatal Diagnosis Unit, Tel Aviv Sourasky Medical Center, the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

³ To whom Correspondence should be addressed; e-mail: dalitb@tasmc.health.gov.il.

improved implantation potential of in vitro fertilized embryos.

The glucose/phosphate-free preimplantation stage 1 (P1) Medium (Irvine, Scientific, Irvine, CA) has been developed on the basis of the fact that embryos apparently grow better in glucose/phosphate-free media (1,4–6). We have routinely used this medium in our IVF unit for in vitro fertilization and embryo development until day 3 (7). Recently, the Cook IVF Medium, a stage-specific family of culture media, was developed by Sydney IVF (Cook IVF, Brisbane, Australia). This prospective randomized trial was conducted to compare embryo development parameters and IVF outcome between the P1 Medium and the Cook IVF Medium.

MATERIALS AND METHODS

The study was performed between November 1999 and April 2000. Inclusion criteria were as follows: patient age <45 years, day 3 FSH level <12 mIU/mL, presence of a normal uterine cavity, as demonstrated by hysterosalpingography (HSG) or hysteroscopy, adequate semen parameters for IVF or ICSI, and the presence of at least 2 follicles ≥ 16 mm in diameter on the day of hCG (human chorionic gonadotrophin) administration. Patients were randomly assigned to the P1 Medium Group (182 patients, 196 cycles) or the Cook IVF Medium Group (167 patients, 179 cycles). Randomization was obtained by a computer-generated randomization table prepared in advance. The randomization assigned one or the other media systems for use during a given day for all patients undergoing oocyte retrieval on that day. The composition of the two commercially available media is detailed in Table I.

Controlled ovarian stimulation was achieved using a short protocol of gonadotrophin-releasing hormone analogue (GnRH-a) and human menopausal gonadotrophin (Pergonal, Teva Pharmaceutical Industries, Ltd.), as described previously (7). Oocyte retrieval was scheduled 35–36 h after administration of 10,000-IU hCG (Chorigon; Teva Pharmaceutical Industries, Ltd.). The cumulus oocyte-complexes were isolated into mHTF (Irvine Scientific) with heparin (P1 Medium Group), or Follicle Flushing Buffer with heparin (Cook IVF Medium Group). Semen preparation was carried out in mHTF supplemented with 5% synthetic serum substitute (SSS) in the P1 Medium Group or Sperm Medium by Irvine Scientific in the Cook IVF Medium Group. Sperm preparation included two cycles of centrifugation (420 g for 10 min)

Table I. Composition of P1 Medium (Irvine Scientific) and Cook IVF Medium (Cook, Australia)

	P1 Medium	Cook IVF Medium
Basic salts	+	+
NaHCO ₃	25 mM	25 mM
Phosphate	—	Low ^a
Glucose		
For fertilization stage	—	+ ^a
For cleavage stage	—	—
Na-pyruvate	0.33 mM	+ ^a
Na-lactate	21.4 mM	+ ^a
Hepes	—	—
Human serum albumin (hSA)	20%SSS is equivalent to 1% hSA	1%
Amino acids	0.05 mM Taurine	Taurine, glutamine, glycine, nonessential amino acids ^a
Na-citrate	0.15 mg/L	—
Phenol red	0.005 g/L	—

^aExact concentrations not provided.

and washing. Then, 300 μ L of the same medium was gently added to the resulting pellet and left in the incubator for 60 min to allow swim up until insemination.

Insemination of the retrieved oocytes was performed using either standard IVF insemination (43% of the cycles) with $\sim 200,000$ motile sperm cells/mL or ICSI (57% of the cycles), according to sperm parameters. If ICSI was performed, the oocytes were denuded of cumulus cells using hyaluronidase and a fine hand-drawn glass Pasteur pipette. Each mature oocyte was placed in 10- μ L droplet of either mHTF with 5% SSS (P1 Medium Group) or Fertilization Medium (Cook IVF Medium Group). Spermatozoa were placed in a 5- μ L droplet of 10% polyvinylpyrrolidone (PVP; Medicult). All droplets were overlaid with paraffin oil (Medicult). ICSI was performed on a Nikon inverted microscope (Diaphot 300) with Narishige micromanipulators. The injected oocytes were than rinsed and returned to the culture dish with either P1 supplemented with 20% SSS (P1 Medium Group) or fertilization medium (Cook IVF Medium Group) for further incubation at 37°C. To ensure optimal culture conditions, CO₂ levels were checked every other day using Normocap 200 (Datex Engstrom). The pH of the media was checked for each batch and the CO₂ level was adjusted for the optimal pH range of 7.2–7.4. Using these criteria, the pH for both media during the study period was within the optimal range. Oocytes were examined for the presence of pronuclei under a dissecting microscope, 18–20-h postinsemination. Embryo cleavage rate and morphology were evaluated on day 2 or 3, prior to transfer. Embryo morphology was scored from 1 to

4 according to the shape of the blastomeres and the amount of detached anuclear fragments. Embryo development was categorized according to cleavage rate into three groups: fast cleaving (>4 cells on day 2 or >8 cells on day 3); normal cleaving (4 cells on day 2 or 8 cells on day 3) or delayed cleaving (<4 cells on day 2 or <8 cells on day 3). Embryos with the highest score were selected for transfer by using a Cook catheter (Cook, Australia). Beginning on the day of embryo transfer, all patients received luteal phase support with 200-mg TID micronized progesterone transvaginally (Utrogestan, Besins Iscovesco, Paris, France).

Serum β hCG levels were measured 12 and 14 days after ET. *Clinical pregnancy* was defined by the presence of a gestational sac, crown rump length, and fetal heart beat at ultrasound performed 6–7 weeks after ET. *Implantation rate* (IR) was defined as the number of gestational sacs divided by the total number of embryos transferred.

Statistical Analysis

The nonparametric Kruskal–Wallis one-way ANOVA test was used for comparing female age, number of retrieved oocytes, fertilization, cleavage, embryo quality, and embryo transfer. χ^2 analysis was used when comparing pregnancy and implantation rates. Numbers are presented as mean $\pm$ standard deviation (SD). A *P* value less than 0.05 was considered as statistically significant.

RESULTS

The study included 349 patients who underwent 375 oocyte retrieval cycles. Patient demographic variables and characteristics are presented in Table II. No sta-

Table II. Patient Demographics

	P1 Medium	Cook IVF Medium	<i>P</i> Value
No. of patients	182	167	
Indication for IVF			ns
Tubal factor	27.9%	19.5%	
Male factor	27.9%	35.5%	
Unexplained	25.7%	20.7%	
Anovulation & endometriosis	4.5%	0%	
Multiple factors	14.0%	24.2%	
Patient age (<i>M</i> $\pm$ SD)	35.2 $\pm$ 6.2	35.4 $\pm$ 5.9	ns
No. of patients >35 years (%)	91 (50%)	83 (50%)	ns
No. of patients >40 years (%)	49 (27%)	46 (27%)	ns
No. of previous failed IVF cycles	4.9 $\pm$ 4.0	5.3 $\pm$ 4.7	ns

Table III. Fertilization and Embryo Development

	P1 Medium	Cook IVF Medium	<i>P</i> Value
Cycles	196	179	
Retrieved oocytes	10.48 $\pm$ 8.2	9.96 $\pm$ 7.6	ns
Fertilization by ICSI	108	107	ns
Fertilization Rate			
2PN fertilization (<i>M</i> $\pm$ SD)	52.3 $\pm$ 26.1%	53.8 $\pm$ 27.6%	ns
1PN fertilization	2.2 $\pm$ 6.1%	2.5 $\pm$ 7.0%	ns
3PN fertilization	4.7 $\pm$ 11.2%	4.0 $\pm$ 10.7%	ns
Cleaved embryos	746	621	
Embryo quality (% of total)			
Grade I	415 (55.7%)	320 (51.5%)	ns
Grade II	215 (28.8%)	203 (32.7%)	ns
Grade III	81 (10.8%)	72 (11.6%)	ns
Grade IV	35 (4.7%)	26 (4.2%)	ns
Developmental rate			
Fast-cleaving embryos (%)	71 (9.5%)	31 (4.9%)	<i>P</i> < 0.0001 ^a
Normal-cleaving embryos (%)	334 (44.8%)	229 (36.9%)	<i>P</i> < 0.0001 ^a
Delayed-cleaving embryos (%)	341 (45.7%)	361 (58.1%)	<i>P</i> < 0.0001 ^a

^a χ^2 test.

tistically significant differences were noted between the two groups regarding indication for IVF, age, and number of previous failed IVF cycles. It is of note that the average age of our patients is 35 years, and as many as 27% are over 40 years of age. Furthermore, many patients have experienced previous IVF failures with an average of five previous failed IVF cycles. In fact, 27% of our patients had five or more previously failed IVF cycles.

Data on mean number of oocytes aspirated, fertilization, embryo quality and cleavage rates are presented in Table III. There were no statistically significant differences between groups regarding the mean number of oocytes retrieved and the proportion of patients requiring ICSI. Likewise, no differences were noted in the rates of normal 2PN fertilization nor in the incidence of 1PN and 3PN fertilization. Embryo quality, as assessed by morphological parameters, was also similar in both groups, with approximately 80% of the embryos being of good quality (grades I and II). However, a significantly higher number of fast- and normal-cleaving embryos were noted in the P1 Medium Group than in the Cook IVF Medium Group (*P* < 0.0001). Conversely, a greater number of delayed cleaving embryos were noted in the Cook IVF Medium Group, than in the P1 Medium Group (*P* < 0.0001).

Embryo transfer, pregnancy and multiple gestation rates, and IRs are described in Table IV.

Table IV. Embryo Transfer Pregnancy Rate

	P1 Medium	Cook IVF Medium	P Value
Cycles	196	179	
Embryo Transfer (ET)			
ET rate	100%	100%	
Total embryos transferred	588	519	
Embryos transferred/cycle	3.0 $\pm$ 1.2	2.9 $\pm$ 1.2	ns
Pregnancy rate (% per ET cycle)			
Positive β hCG/ET	54 (27.6%)	36 (20.1%)	ns
Clinical pregnancy/ET	38 (19.4%)	23 (12.8%)	ns
Delivery rate/ET	32 (16.3%)	20 (11.1%)	ns
Multiple pregnancy			
Twin	12 (31.5%)	4 (17.4%)	ns
Triplet	4 (10.8%)	2 (8.7%)	ns
Total	16 (42.1%)	6 (26.1%)	ns
Implantation rate	58/588 (9.9%)	31/519 (6%)	$P \leq 0.025^a$

^a χ^2 test.

Normally cleaving embryos were available for transfer in all cycles in both groups. While the mean number of embryos transferred in both groups was similar, all IVF outcome parameters were more favorable in the P1 Medium Group, albeit not statistically significant. The rate of positive β hCG in the P1 Medium Group was 29.1% compared to 21.3% in the Cook Medium Group. Likewise, higher clinical pregnancy rates (20.3% vs. 13.6%, respectively) and higher delivery rates (17% vs. 11.8%, respectively) were noted in the P1 Medium Group than in the Cook IVF Medium Group. The multiple pregnancy rate was also higher in the P1 Medium Group, but this too was not a significant difference. Finally, the implantation rate was significantly higher in the P1 Medium Group than in the Cook IVF Medium Group (9.9% vs. 6%, respectively, $P = 0.025$).

DISCUSSION

Success rates in IVF are influenced by a multitude of factors, the most important of which are patients' age, number of oocytes retrieved per cycle, and the quality of embryos transferred. Since the latter may be influenced by the culture conditions, clinicians and researches have never ceased to search for optimal embryo culture media. It has been suggested that in vitro development of human embryos can be achieved using a wide variety of culture media which differ mainly in glucose and phosphate concentrations, variety of amino acids inclusion, type of serum added,

and whether or not a pH indicator (phenol red) is added.

A previous study performed in our center provided data favoring the use of the P1, glucose-free, medium supplemented with 20% SSS (7). We have since used this culture system in our IVF program. Recently, however, "new generation" commercial culture media have become available. It has been claimed that these improved culture conditions would yield more viable embryos with a better implantation potential. Of these, the Cook IVF Medium was selected especially because it is glucose-free from the 2PN stage. The purpose of the study was to perform a prospective randomized comparison between the P1 Medium and the Cook IVF Medium.

All variables that might have had an impact on IVF outcome, such as the indication for IVF, patients' age, number of previous IVF attempts, the proportion of cycles with ICSI, the number of oocytes aspirated, as well as the number of embryos transferred, were similar in both the P1 Medium Group and the Cook IVF Medium Group. Likewise, the pH for both media during the study period was within the optimal range, and at CO₂ levels between 5.2 and 5.6%, the pH of the Cook was only 0.15 $\pm$ 0.02 higher ($M \pm SD$) than that of the P1 of Medium. Thus any differences in developmental rate, embryo quality, pregnancy rates, and implantation rates may be attributed to the differences in the media characteristics. Indeed, a significantly higher proportion of fast- and normally cleaving embryos was noted in the P1 group while a significantly greater proportion of delayed-cleaving embryos was observed in the Cook IVF Medium Group. Collectively, these data suggest significant difference in the developmental rate between the two media systems. Likewise, De Clerck *et al.* (8) found that embryo cleavage was retarded in Cook IVF Medium as compared to Menezo B2 medium. Similar results of embryos displaying faster early developmental kinetics were observed with the G1.2 medium as compared to Sydney IVF Medium (9). This observation may be important because fast-cleaving embryos have previously been shown to have a higher implantation potential in animal species (10).

Furthermore, fast-cleaving human embryos demonstrated a greater potential to develop to blastocysts in vitro (11). In a previous study of 271 IVF/ICSI cycles we have demonstrated that fast-cleaving embryos have a higher implantation potential than normal or delayed ones (unpublished results). In an elective single-embryo transfer program, early cleaved human embryos had a significantly higher developmental

competence than delayed cleavage embryos (12). In addition, pregnancy and implantation rates were higher in patients who demonstrated early cleaving embryos (13). Our results suggest that culture in P1 Medium yields faster developing embryos than in the Cook IVF Medium. The P1 Medium was also associated with a significantly higher implantation rate than the Cook IVF Medium. While pregnancy rates were more favorable with the P1 Medium, this did not reach statistical significance. If the same rates applied for a study group 50% greater in size, the differences would have been statistically significant. However, once the data were analyzed, we felt it would be ethically unjustified to increase the size of the study group, given the apparently better results with the P1 Medium. Suffice it to say that the Cook IVF Medium is certainly not associated with improved results.

What could account for the different outcomes observed with the P1 Medium versus the Cook IVF Medium? One possibility is the addition of glucose to the Cook IVF Medium in the fertilization stage. Nonetheless, no differences in fertilization rates were observed between the two groups. Another major difference between the media is the composition of the serum additives. However, this parameter is difficult to interpret as the precise composition is not disclosed. When SSS is added at 20% to the P1 Medium, it is roughly equivalent to 1% human serum albumin (hSA) present in the Cook IVF Medium. However, SSS contains 6% proteins, (only 5% of which is pharmaceutical grade human serum albumin and ~1% human globulins heat-treated at 60°C). The presence of globulin with hSA has been shown to enhance the rate of embryo development and pregnancy rates beyond the levels achieved for hSA alone (14,15).

In conclusion, our data show that the Cook IVF Medium is definitely not superior to the P1 Medium, and in fact, the contrary may hold true. This underlies the importance of the continuous search for optimal culture media in IVF.

ACKNOWLEDGMENT

E. Eshkol is thanked for editorial assistance.

REFERENCES

1. Gardner DK, Lane M, Calderon I, Leeton J: Environment of the preimplantation human embryo in-vivo: Metabolite analysis of oviduct and uterine fluids and metabolism of cumulus cells. *Fertil Steril* 1996;65:349–353
2. Karamalegos C, Bolton VN. A prospective comparison of “in house” and commercially prepared Earle’s balanced salt solution in human in-vitro fertilization. *Hum Reprod* 1999;14:1842–1846
3. Conaghan J, Handyside AH, Winston RM, Leese HJ. Effects of pyruvate and glucose on the development of human preimplantation embryos in vitro. *J Reprod Fertil* 1993;99:87–95
4. Bavister BD. Culture of preimplantation embryos: Facts and artifacts. *Hum Reprod Update* 1995;1:91–148
5. Barnett DK, Bavister BD. Inhibitory effect of glucose and phosphate on the second cleavage division of hamster embryos: Is it linked to metabolism? *Hum Reprod* 1996;11:177–183
6. Coates A, Rutherford AJ, Hunter H, Leese HJ. Glucose-free medium in human in vitro fertilization and embryo transfer: A large-scale, prospective, randomized clinical trial. *Fertil Steril* 1999;72:229–232
7. Ben-Yosef D, Yovel I, Schwartz T, Azem F, Lessing JB, Amit A. Increasing synthetic serum substitute (SSS) concentrations in P1 glucose/phosphate-free medium improves implantation rate: A comparative study. *J Assist Reprod Genet* 2001;18:588–592
8. De Clerck E, Janssens R, Joris H, Verheyen G, Van Steirteghem A: Prospective, controlled, randomized comparison of two embryo culture systems: Menezo B2 medium and Cook IVF medium. *In ESHRE Annual Meeting, Bologna, Italy, 2000*, pp. 801–900
9. Van Langendonck A, Demylle D, Wyns C, Nisolle M, Donnez J. Comparison of G1.2/G2.2 and Sydney IVF cleavage/blastocyst sequential media for the culture of human embryos: A prospective, randomized, comparative study. *Fertil Steril* 2001;76:1023–1031
10. McKiernan SH, Bavister BD. Timing of development is a critical parameter for predicting successful embryogenesis. *Hum Reprod* 1994;9:2123–2129
11. Muggleton-Harris AL, Glazier AM, Wall M. A retrospective analysis of the in-vitro development of “spare” human in-vitro fertilization preimplantation embryos using “in-house” prepared medium and “Medi-Cult” commercial medium. *Hum Reprod* 1995;10:2976–2984
12. Salumets A, Hyden-Granskog C, Makinen S, Suikkari AM, Tiitinen A, Tuuri T. Early cleavage predicts the viability of human embryos in elective single embryo transfer procedures. *Hum Reprod* 2003;18:821–825
13. Isiklar A, Mercan R, Balaban B, Alatas C, Aksoy S, Urman B. Early cleavage of human embryos to the two-cell stage. A simple, effective indicator of implantation and pregnancy in intracytoplasmic sperm injection. *J Reprod Med* 2002;47:540–544
14. Pool TB, Martin JE. High continuing pregnancy rates after in vitro fertilization–embryo transfer using medium supplemented with a plasma protein fraction containing alpha- and beta-globulins. *Fertil Steril* 1994;61:714–719
15. Weathersbee PS, Pool TB, Ord T. Synthetic Serum Substitute (SSS): A globulin-enriched protein supplement for human embryo culture. *J Assist Reprod Genet* 1995;12:354–360