Embryo quality and implantation rate in two different culture media: ISM1 versus Universal IVF Medium

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Objective: To compare the outcome of two different culture media marketed by the Medicult AS Company (Jyllinge, Denmark)—Universal IVF Medium and ISM1 Medium culture—which, in addition to glucose, pyruvate, and energy-providing components, also contain amino acids, nucleotides, vitamins, and cholesterol.

Design: Laboratory and retrospective clinical study.

Setting: University teaching hospital.

Patient(s): A total of 726 patients, undergoing IVF–intracytoplasmic sperm injection procedure, comparable in mean age range, oocyte retrieval, and infertility indication, were included in the study. Laboratory quality and standard procedures were maintained unaffected.

Intervention(s): Oocyte retrieval, different embryo culture media.

Main Outcome Measure(s): Embryo quality, ongoing pregnancy, and implantation rate.

Result(s): The frequency of good-quality embryos (79% vs. 74%) and the percentages of ongoing pregnancy (27.5% vs. 18%) and implantation rate (15% vs. 10%) were significantly higher in the group treated with ISM1 Medium rather than Universal IVF Medium.

Conclusion(s): ISM1 Medium culture seems to improve the performance of embryonic growth and development, as well as increasing the percentage of pregnancy. (Fertil Steril 2010;93:1859–63. ©2010 by American Society for Reproductive Medicine.)

Key Words: Culture medium, embryo metabolism, imprinting, implantation rate

After three decades approximately 3 million children have been born worldwide through use of IVF (1). Numerous efforts and studies have been made to offer the couples undergoing assisted reproduction technology (ART) treatments an increased success rate and a decreased number of multiple pregnancies.

In addition to a number of innovations that have been introduced in ART laboratory technologies (e.g., assisted hatching, intracytoplasmic morphologically selected sperm injection (IMSI) analyses, the use of polarized light field microscopy), progress also has been made in the field of culture media to guarantee a greater success rate. However, numerous aspects concerning embryo needs and culture conditions still require elucidation.

There are numerous different in vitro culture systems available for laboratory procedures applied to gamete maintenance and embryonic growth. These media are widely used, from the simplest solutions to the more complex culture media, and many recent discoveries have been fundamental in describing different biochemical, physiologic, genetic, and epigenetic characteristics of the embryo, leading to recent innovations in this field. Culture media formulations are considered to be of fundamental importance in embryo culture laboratory procedures, and their influence has been reviewed in several studies (2–4).

Since the first media used, formulated from simple components, such as balanced salt solutions (e.g., Earle’s, human tubal fluid [HTF], T6) (4, 5) and generally supplemented with whole serum or with serum albumin, significant changes have occurred to satisfy a progressive increase in embryo metabolic demands and the aim of maintaining inherited embryo viability. The use of monoculture media revealed an embryonic capacity to adapt to a stable environment and to base the nutritional source on a single medium. In these conditions embryo growth could be affected because the lack of many nutrients forces embryos to expand significant energy resources (4). When it became clear that during development the human embryo changes its energy needs, more sensitive and specific culture media immediately were designed. It has been demonstrated that in the initial phases of development, when the early embryo is under maternal genetic control, pyruvate and lactate are used as energy substrates. In the subsequent phases, when the embryonic genome has been activated, the metabolism is dependent on glucose consumption (2). The use of culture media must be appropriate for the developmental
stage of the embryo. During the early cleavage stage it is important to minimize stress mechanisms and to regulate gene expression and methylation patterns to allow correct development up to the blastocyst stage. Subsequently it is important to guarantee appropriate support for blastocyst maintenance and implantation (4). For these reasons it has become necessary to introduce sequential and more specific media to satisfy embryo needs during each phase of development. The G1 and G2 media (Vitrolife, Göteborg, Sweden), created and elaborated by Gardner, are based on the consideration that during the early embryo developmental phase only a few amino acids are required, whereas in the compaction phase a full set of 20 amino acids is needed (6).

Many systems have been developed since the advantages of the application of sequential media were recognized. Widely used in Europe are MediCult ISM1 and ISM2 media (MediCult, Jyllinge, Denmark), developed by Menezo, including components such as glucose and derived metabolites, amino acids, nucleotides, vitamins, and cholesterol. In this way, a sequential medium is designed to imitate the micro environment between oviduct and uterus and to reduce culture stress in the in vitro condition, where thermal, metabolic, osmotic, and oxidative shocks could occur (7).

Nevertheless, although great effort has been invested in improving culture media, there have been few studies involving a comparison of sequential versus monoculture media. In the present study we wanted to assess the correlation between embryonic development and implantation and the use of two different culture media: Universal IVF Medium (MediCult), a simple monoculture medium that contains glucose, pyruvate, and energy-providing components, and ISM1 Medium, a sequential medium also enriched with amino acids, nucleotides, vitamins, and cholesterol.

MATERIALS AND METHODS

A total of 726 patients (323 in the Universal IVF Medium group and 403 in the ISM1 Medium group) who underwent treatment from March 2004 to March 2008 were included in this retrospective study. The inclusion criteria were counseling patients undergoing IVF–intracytoplasmic sperm injection (ICSI) treatment, age 22 to 46 years, and fertilizing patients undergoing IVF–intracytoplasmic sperm injection procedure. During the examined period Universal IVF Medium was used between March 2004 and March 2006, then replaced with ISM1 Medium between March 2006 and March 2008. For clarity all laboratory procedures were maintained constant. All patients received pharmacologic ovarian stimulation for ART. Patients with infertility diagnosis of tubal factor, male factor, unexplained infertility, and ovulatory disorders were included.

Ovarian Stimulation, Oocyte Retrieval, and Culture Protocol

Pharmacologic stimulation was achieved with use of a combination of a GnRH analogue (Enantone 3.75 or Enantone die 0.2; Takeda, Tokyo, Japan) and gonadotropins (Gonal F; Serono, Geneva, Switzerland; or Puregon; Organon, NV, OSS, The Netherlands), in accordance with a long standard protocol. The FSH dose was administered in relation to the age of the patient (e.g., < 30 years: 150 IU SC per day; 30–37 years: 225 IU SC per day; >37 years: 400 IU SC per day). The dose then was modified on the basis of an ultrasound check on an individual basis. Follicular growth was monitored by ovarian ultrasonography. Ovulation was induced with 10,000 IU of hCG (Gonasi; AMSA, Rome, Italy) when at least two follicles of ≥18 mm in diameter were observed. Transvaginal ultrasound–guided oocyte retrieval was performed 34 to 36 hours after the hCG administration.

The oocytes were checked in the follicular aspirates and washed in 3 mL Game medium (Vitrolife) separated from their follicular fluid, and each of them singularly was transferred to a 50-μL microdrop of Universal IVF Medium under paraffin oil and incubated at 37°C in an atmosphere of 5% CO₂ in air until the insemination procedure.

Sperm Preparation by Sperm Swim-Up

Semen was placed in a Falcon tube (BD Biosciences, Franklin Lakes, NJ), washed, and centrifuged (1,200 rpm) before the culture medium (Sperm Rinse; Vitrolife) was carefully placed on top of the sperm to induce selection, and the sperm suspension was kept in a 37°C incubator until IVF or ICSI of the oocytes was performed.

Fertilization, Cleavage, and ET

Once inseminated (a maximum of three oocytes per patient according to Italian law regulating ART procedures) the oocytes were returned to 50-μL microdrops of Universal IVF Medium and cultured at 37°C in an atmosphere of 5% CO₂ in air. The oocytes were examined between 16 and 20 hours after insemination to determine the presence of two pronuclei and the extrusion of the second polar body. Laboratory procedures were standardized and remained constant during both analyzed periods with the exception of the culture media: in 323 patients (group 1) Universal IVF Medium was used as monoculture medium from oocyte retrieval until ET; in 403 patients (group 2) Universal IVF Medium was replaced by ISM1 Medium culture from fertilization check (for both IVF and ICSI procedures) to ET procedure.

Each normally fertilized oocyte was transferred to a new 50-μL microdrop of preequilibrated Universal IVF Medium in group 1, and a 50-μL microdrop of preequilibrated ISM1 Medium culture in group 2. Embryos were kept in the same medium from fertilization check until day 3, and therefore no extra media changes were needed for day 3 transfer.

Embryos were scored from the best to the worst, graded A, B, C, D on day 3 according to developmental rate and morphologic quality (8). Embryo grading was based on morphologic appearance and embryo development. Morphologic parameters considered were size regularity, shape of blastomeres, and presence or absence of cytoplasmic vacuoles, granulations, and extracellular fragments. Embryos showing a normal cleavage rate, regular blastomeres, absence of
fragmentation, and a clear, bright, and homogeneous cytoplasm were scored as grade A; embryos with irregular blastomeres and/or slow development, <10% of fragmentation, and slightly granulous cytoplasm were scored as grade B; embryos that showed dark cytoplasm and fragmentation <30% and >30% with or without a very slow development were scored as grades C and D, respectively. Embryos exhibiting a slow development under morphologic observation were down-scored by one grade level (8).

An ET procedure was performed at day 3, approximately 72 hours after oocyte retrieval, with use of the same medium for all patients (EmbryoGlue Medium; Vitrolife). Transcervical transfer was carried out with use of a Cook K-Soft 5000 catheter (Cook, Melbourne, Australia).

Intramuscular P was administered as a luteal support until the b-hCG assay was performed. The implantation rate was defined as the number of gestational sacs per transferred embryo, and the abortion rate was calculated in the third trimester of pregnancy. Only pregnancies with ultrasonographic evidence of fetal heart activity at 7 weeks were considered as clinical pregnancies.

Statistical Analysis
Comparison between the groups was calculated by χ² test and by paired t-test. The data were presented as mean ± SD unless otherwise indicated. P < .05 was considered statistically significant.

RESULTS
The patients’ mean age was similar for both groups (Table 1). Both groups had similar percentages of patients with tubal disease, male factor, anovulation, and idiopathic causes of infertility (Table 1).

Moreover, the mean number of retrieved oocytes, mature oocytes, and fertilization rate was similar in both groups of patients. The percentage of good-quality cleaved embryos (with A or B score) was significantly higher in group 2 than in group 1 (79% vs. 74%, P < .005) (Table 2).

The ISM1 Medium group resulted in a statistically significant higher rate of clinical pregnancies (31% vs. 21%, P < .005) and an implantation rate of 15% versus 10% (P < .005) compared with the Universal IVF Medium group, whereas there was no statistically significant difference in the abortion rate in the first trimester, 11% versus 15% between the two groups. Furthermore, statistically, ongoing pregnancies were significantly higher in the ISM1 Medium group than in the Universal IVF Medium group at 27.5% versus 18% (P < .005).

DISCUSSION
This study suggested that ISM1 Medium appears to be more efficient in improving embryo quality with respect to IVF Universal Medium during the in vitro culture period. These findings are supported by a statistically significantly higher frequency of good-quality embryos and a statistically significantly higher percentage of clinical pregnancies, implantation rate, and ongoing pregnancy rate.

On the basis of these considerations, it was possible to assess that Universal IVF Medium, used as a monoculture medium, should be replaced by ISM1 Medium after the fertilization check, with the supplemented ISM1 components seeming to better sustain in vitro embryo growth. The effectiveness of different commercial culture media for in vitro embryo culture was evaluated: some authors found no differences in terms of embryo quality or pregnancy or implantation rate (9–11), whereas others observed a slight discrepancy in day 3 embryo quality between Vitrolife G1.2 and Sydney IVF sequential media (12), as well as between Vitrolife G1.2 and HTF medium (13). In the most recent study, in which a comparison was made between three different commercial IVF media and an HTF medium produced “in house,” the authors demonstrated that, on day 3 of the embryo culture, both Life Global and Sage Cleavage media exhibited a similar effectiveness in terms of embryo quality and implantation and pregnancy rate, whereas...
metabolic requirements change (4). On the basis of these ob-
late blastocyst phase. At the eight-cell stage, embryonic DNA
and pyruvate as energy-providing components, which are
Conversely, Universal IVF Medium, used as a monoculture
and antioxidant defense, energetic metabolite (Na,
consumption, the balance between reactive oxygen species
in vivo culture the lack of this system could be responsible
during the early embryonic development phases up to the blastocyst
stage, later increasing (15). Furthermore, it has been hypoth-
esized that oxidative stress induces embryos into a “senes-
cence-like” state, and it has been observed that, in vivo,
gamete development also is due to a delicate balance between
reactive oxygen species and the antioxidant system. Al-
though in vivo physiologic reactive oxygen species produc-
tion is controlled continuously by a strong cellular defense,
in vitro culture the lack of this system could be responsible
for several consequences including the oxidation of proteins,
lipids, DNA, and the misregulation of metabolic pathways
that may lead to cell death (16–18). On the basis of these ob-
servations, it can be speculated that the antioxidant elements
contained in ISM1 Culture Medium sustain the cultured em-
bryos thereby avoiding developmental failure and also
achieving the best quality for implantation.

Considering the energetic metabolites and amino acid
turnover, it was established that the activity of some enzymes
(e.g., adenosine triphosphatase) and the intense synthesis of
some molecules (DNA, RNA, proteins, and lipids) are re-
ponsible for the marked increase in oxygen consumption
and for the different amino acid expression, particularly at
the blastocyst stage (15). It also was observed that embryos
exhibiting a strong metabolic system with a higher amino
acid turnover appear to develop better that those affected
by a form of metabolic stress (15). In fact, amino acids
have proved fundamental in the imprinting process essential
for embryo development and implantation capability. A

**TABLE 2**

**Cycle characteristics according to the embryo culture medium used.**

<table>
<thead>
<tr>
<th>Universal IVF Medium (n = 323)</th>
<th>ISM1 Medium (n = 403)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of oocytes</td>
<td>7.14 ± 4.6</td>
<td>6.67 ± 3.9</td>
</tr>
<tr>
<td>No. of mature oocytes (metaphase II)</td>
<td>5.52 ± 3.78</td>
<td>4.92 ± 3.17</td>
</tr>
<tr>
<td>Fertilized oocytes (n) (%)</td>
<td>798/902 (88)</td>
<td>1,075/1,189 (90)</td>
</tr>
<tr>
<td>Good-quality embryo (A + B score) (n) (%)</td>
<td>577/784 (74)</td>
<td>845/1,065 (79)</td>
</tr>
<tr>
<td>No. of embryos transferreda</td>
<td>2.43 ± 0.76</td>
<td>2.64 ± 0.6</td>
</tr>
<tr>
<td>Clinical pregnancies (n) (%)</td>
<td>67/323 (21)</td>
<td>125/403 (31)</td>
</tr>
<tr>
<td>Implantation rate (n) (%)</td>
<td>80/778 (10)</td>
<td>166/1,075 (15)</td>
</tr>
<tr>
<td>Abortion rate (n) (%)</td>
<td>10/67 (15)</td>
<td>14/125 (11)</td>
</tr>
<tr>
<td>Ongoing pregnancies (n) (%)</td>
<td>57/323 (18)</td>
<td>111/403 (27.5)</td>
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*Note: NS = not significant.
Values are mean ± SD.


a significant difference was observed between Sage Cleavage
and in-house HTF media only for IVF embryo quality. More-
over, Vitrolife G1.2 appeared less effective for ICSI embryo
quality compared with in-house HTF medium. These findings
suggested that a difference between available commercial and
in-house–produced media may exist, particularly in terms of
different batch and substance variations (e.g., synthetic or
human serum) (14).

In addition to these observations, the possibility of mixed
ET (cultured under different culture conditions) may limit
the validity of the above conclusions. In our study, albeit lim-
ted to a retrospective analysis, it was possible to ensure the
effectiveness of transferred embryos in each of the specific
culture media, confirming the effectiveness of a richer
medium such as ISM1 Medium.

MediCult ISM1 Culture Medium, developed by Menezo,
was created with the idea that components such as glucose
and derived metabolites, amino acids, nucleotides, vitamins,
and cholesterol could be helpful for embryo completion.
Conversely, Universal IVF Medium, used as a monoculture
medium, is a much simpler medium containing only glucose
and pyruvate as energy-providing components, which are
very important for the earlier phases of embryo development
but probably not sufficient for the later phases.

During its journey from the tubes through the oviduct
fluids to the uterus floor, the embryo undergoes many
changes, in terms of cell division, genomic asset activation,
and required nutrients. The studies that investigated embryobiosis
metabolism from the early preimplantation phases to the
morula-blastocyst implantation stage showed how some sub-
stances detrimental to early embryos become beneficial in the
late blastocyst phase. At the eight-cell stage, embryonic DNA
is activated and a correct genetic imprinting is needed as the
metabolic requirements change (4). On the basis of these ob-
servations and on Gardner’s considerations about the specific
phase of embryo growth, new specific sequential media were
created (6).

In vivo, as well as in vitro, fundamental factors for proper
embryonic development could be summarized as oxygen
consumption, the balance between reactive oxygen species
and antioxidant defense, energetic metabolite (Na,
adenosine triphosphatase), and amino acid turnover. The ox-
ygen requirement appears constant over the course of the
early embryonic development phases up to the blastocyst
stage, later increasing (15).
recent study reported that serum-free sequential media showed correct genetic imprinting when compared with two different monoculture media showing a high percentage of incorrect imprinting (19).

On the basis of these observations, ISM1 Culture Medium was formulated to promote correct imprinting to ensure correct development and implantation. As demonstrated by Cassuto et al. (7), different timing and culture conditions can have different effects on embryo development and implantation rate. Extended culture time and particular culture conditions (ISM1/ISM2 vs. ISM1/M3 culture media [Incell, San Antonio, TX]/ISM2) can result in an increase in monozygotic twinning. In this study the use of only ISM1/ISM2 culture conditions resulted in lower monozygotic twinning pregnancy, independently of the technology used (IVF/ICSI) (7).

To our knowledge, only a few studies have investigated the effect of ISM1 Medium on embryo quality and implantation rate. A prospective study on sibling oocytes was carried out to compare the effects of FertiCult (FertiPro, Beernem, Belgium) versus ISM1 culture media on embryo quality in the day 2 stage. The percentage of good-quality embryos (A+B) was 53.2% versus 64.5% in IVF procedures (P<0.03) and 59.4% versus 76.8% in ICSI procedures (P<0.0001), showing that embryo quality in the day 2 stage appeared enhanced under ISM1 culture rather than FertiCult culture medium, even if data regarding pregnancy and implantation rate were not reported (20).

Our study supports this finding, also demonstrating how both pregnancy and implantation rates are significantly improved by the introduction of ISM1 Medium in embryo culture. Like many other sequential media designed to mimic the maternal micro ambient to reduce thermal, metabolic, osmotic, and oxidative shocks that might increase stress in in vitro culture conditions, ISM1 Medium seems to facilitate embryo growth, guaranteeing a higher pregnancy and implantation rate. Despite the circulation of new, innovative research and findings, some issues concerning embryonic needs and culture conditions still need to be clarified.

REFERENCES