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 expend 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

Received July 23, 2008; revised December 2, 2008; accepted December 11, 2008; published online January 18, 2009.
S.X. has nothing to disclose. T.M. has nothing to disclose. D.T. has nothing to disclose. S.G. has nothing to disclose. A.L.M. has nothing to disclose. A.T. has nothing to disclose. A.V. has nothing to disclose.
Supported with a grant from the Fondazione Cassa di Risparmio di Modena and FIRB.
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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, Goteborg, 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 a minimum of two follicles (>18 mm) in the oocyte collection 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 $\geq$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 Gamete 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% CO_2 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% CO_2 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