Use of a Medium Devoid of Any Human or Animal Compound (SMART2¹) for Embryo Culture in Intracytoplasmic Sperm Injection

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Purpose: Our purpose was to evaluate the efficiency of a medium, devoid of any human or animal compound and specially designed for early embryo development (from the zygote to the eight-cell stage), SMART2, in intracytoplasmic sperm injection (ICSI) and to compare it with a medium containing human serum albumin (EllioStep2).

Methods: Oocytes from 50 ICSI attempts were randomly placed, after sperm injection, into either SMART2 or EllioStep2. After a 48-hr incubation, the embryos were examined for quality scoring before transfer or freezing.

Results: The percentage of normally fertilized oocytes per intact oocytes was slightly higher using SMART2 (139/199 vs. 135/224, respectively, for SMART2 and EllioStep2; $P < 0.05$). The distribution of embryo scores and the percentage of embryos with a fair morphology (71/143 vs. 72/148, respectively, for SMART2 and EllioStep2; not significant) were identical in both media.

Conclusions: These data show that SMART2 medium can be successfully used for early embryo growth and, because it is devoid of any human or animal compound, offers better safety for patients than conventional media.

KEY WORDS: human; medium; ICSI; embryo.

INTRODUCTION

Culture media used for assisted reproductive technologies are usually supplemented with serum (1) or serum-extracted proteins such as human serum albumin (HSA) (2) or bovine serum albumin (BSA) (3). The use of such supplementation raises two problems:

reproducibility and safety. Indeed, it has been shown that some sera could have detrimental effects on embryo growth (4,5) and that there are wide batch-to-batch variations among albumin preparations (6,7). To solve this problem, standardized serum substitutes, which contain proteins extracted from pooled human sera (8), have been successfully used in in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) (9,10). The safety problem is related to the ability of pathogens, such as viruses, to bind to plasma proteins and, especially, to albumin (11,12). Despite the efficiency of the preparation processes in eliminating known pathogens, it is impossible to eliminate hazards completely due to unknown ones (13).

These data have led us to formulate a medium devoid of any human or animal compound, SMART2 (Bio-Média, Boussens, France). Since the nutrient requirements of fertilization and early and late preimplantation embryo development are very different (14–17), we designed this medium especially for the embryonic growth from the zygote to the eight-cell stage. We report here the results of a comparison, in ICSI, of SMART2 and EllioStep2 (Ellios Bio-Media, Paris, France), a medium also designed for early embryo growth but containing HSA (18).

MATERIALS AND METHODS

Media

SMART2 is a complex medium containing low levels of glucose, high levels of pyruvate, EDTA, nonessential amino acids, and vitamins. It is devoid of any human or animal compound. Instead of proteins it contains dextran and plant molecules.

EllioStep2 is a complex medium containing low levels of glucose, high levels of pyruvate, EDTA, nonessential amino acids, and vitamins. It also contains 1% HSA.

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Because neither SMART1 nor EllioStep2 is designed for sperm capacitation and gamete interaction, ICSI was performed in BM1 medium (Ellios Bio-Media, Paris, France), which is a complex medium with high levels of glucose and low levels of pyruvate and without EDTA. This medium also contains 1% HSA.

Patients

Fifty patients undergoing ICSI for failed or very low fertilization rate in a previous IVF cycle, or sperm parameters too poor for IVF, or epididymal sperm retrieval entered the study.

Ovarian stimulation was performed using a combination of buserelin (Suprefact, Hoechst, Puteaux, France) and recombinant follicle-stimulating hormone (FSH) (Puregon 150; Organon, Paris, France) as previously described (19). Three to 18 oocytes (mean, 9.9) were collected by ultrasonically guided puncture.

Oocyte Preparation

After collection, oocytes were pooled in BM1 medium and incubated at 37°C with 5% CO₂ until denudation. Cumulus-oocyte complexes were treated with hyaluronidase, 80 IU/ml, in Earle's medium (Sigma, St. Louis, MO). Cumulus-oocytes complexes were placed in a 100- μ l droplet of the enzyme solution for cumulus removal. They were then placed in a 100- μ l droplet of BM1 and corona was removed using a yellow tips (Gilson, Villiers-le-Bel, France). The oocytes were then rinsed twice in BM1 medium and incubated in BM1 medium until sperm injection.

ICSI Procedure

The ICSI procedure was identical to the one described by Palermo *et al.* (20). The pipettes were obtained from Cook France (Charenton, France) and PVP from Scandinavian Science (JCD, Gauville, France). After sperm injection, oocytes were randomly placed in SMART2 or EllioStep2 and incubated at 37°C with 5% CO₂. At 16–18 hr after injection, oocytes were examined for survival and fertilization. Embryo quality was evaluated 40 hr after injection according to the score described by Giorgetti *et al.* (21), and one to four embryos (mean, 2.9) were transferred according to patient's age.

Statistical Analysis

Data are means $\pm$ standard deviation. Statistical comparisons were performed using the χ^2 test with Yates' correction.

RESULTS

As shown in Table I, the normal fertilization (two pronuclei) rate per intact oocytes was slightly higher when SMART2 was used. The percentages of activated oocytes (1PN) and of triploid zygotes (3PN) were identical in both media. The distribution of the embryo scores and the percentage of embryos with a fair morphology (scores 3 and 4) (50 vs. 49%, respectively, for SMART2 and EllioStep2; not significant) were also identical. Since the randomization was done on an oocyte and not on a patient basis, the pregnancy rates could not be compared between media. However, in 12 cases, only embryos obtained with SMART2 were transferred, allowing us to obtain four clinical pregnancies (vs. 5/15 with only embryos obtained in EllioStep2 and 6/23 with embryos from both SMART2 and EllioStep2).

DISCUSSION

The results of the present study show that, despite the absence of serum protein, SMART2 is as efficient as EllioStep2 in supporting early embryo growth.

Table I. Results of ICSI as a Function of the Medium Used After Sperm Injection

	SMART2	EllioStep2
No. of injected oocytes	236	257
No. of intact oocytes (% injected oocytes)	199 (84)	224 (87)
No. of 2 PN ^a (% intact oocytes)	139 (70)*	135 (60)
No. of 3 PN (% intact oocytes)	6 (3)	5 (2)
No. of 1 PN (% intact oocytes)	3 (2)	7 (3)
No. of embryos (% intact oocytes)	143 (72)	148 (66)
Embryo score (%)		
4	20 (14)	20 (14)
3	51 (36)	52 (35)
2	46 (32)	54 (36)
1	25 (17)	22 (15)

^a Pronuclei.

* $P < 0.05$ vs. EllioStep2.

SMART2 medium was compared with EllioStep2 medium because both media are especially designed for early embryonic growth from the zygote to the eight-cell stage. Indeed, the metabolism and thus the nutrient requirements of the fertilization and preimplantation embryonic development are very different and sometimes contradictory. For instance, glucose is the essential source of energy for spermatozoa and high concentrations are required for fertilization (22). On the other hand, glucose is not used by the embryos up to the eight-cell stage and can even be detrimental for their development (14,23,24). However, glucose is needed again to reach the blastocyst stage (14). These differences found for glucose exist for a wide variety of molecules such as amino acids and chelating agents (15–17). Therefore, there is a benefit of using sequential media in IVF instead of general media. Since we have previously reported that EllioStep2 gave better results in ICSI than IVF50 (Scandinavian IVF Science, Gothenburg, Sweden) and BM1 (18), we have chosen to compare SMART2 to it. As SMART2 and EllioStep2 have given similar results, this study demonstrates that serum proteins, especially albumin, are not necessary to support embryonic development and can be replaced by totally synthetic molecules. This finding has an important impact on the routine practice of assisted reproductive technology, because by avoiding serum proteins, we alleviate potential hazards due to their use. Indeed, viruses can bind to serum proteins, and since they have a great variability (25), it is quite impossible to ensure a complete safety with extracted molecules. Moreover, the emergence of new kind of pathogens, such as prions, increases the difficulties in assessing the absence of any infectious risk with human or animal extracted proteins. Another advantage of using protein-free media is the batch-to-batch reproducibility. Indeed, the variations among batches lead to test each of them on mouse embryo system prior to use (26,27). These variations are essentially due to the protein content of the media, since Kane (7) in rabbits, Batt and Miller (28) in sheep, and McKiernan and Bavister (29) in hamsters have reported that the percentage of embryos reaching the blastocyst stage was greatly influenced by the batch of albumin used to supplement the culture media. On the other hand, Kane (7) found no influence of the batches of amino acids. Dröge *et al.* (6) have shown that the main differences between albumin lots concerned their fatty acid content, which have a great influence on embryo development. Indeed, palmitic acid and polyunsaturated fatty acids inhibit mouse embryo development through the generation of superoxide radicals (30). Such an

influence of the variability of the fatty acid content of albumin lots is a common problem in tissue culture and has been reported in other biological systems such as Leydig cell steroid production (31).

This study was performed in ICSI to avoid any interference with fertilization because SMART2 is not designed for gamete interaction. Therefore SMART2 cannot be used as a unique medium for assisted reproductive technologies and must be used in a second step after fertilization in a medium such as SMART1 (Bio-Média). This latter medium, also devoid of any human or animal compound, is able to support the first steps of assisted reproductive technologies (32).

In conclusion, since SMART2 allows a good efficiency with a high safety and reproducibility, it can be used in ICSI to culture oocytes just after sperm injection and in IVF to incubate zygotes after the first 24 hr in the insemination medium.

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