Simultaneous culture of embryos in two separate sequential media systems improves pregnancy outcomes.

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I ABSTRACT

OBJECTIVE: The effect of simultaneously culturing an individual patient's embryos in more than one sequential media system was examined.

DESIGN: Retrospective data analysis of two sequential media systems for culturing embryos. From January through October 2004 (Pre-treatment phase) all embryos were cultured in a single media series. From November 2004 to the present (Treatment phase) embryos were evenly divided between two separate manufacturer's media system and the best embryos chosen for transfer.

MATERIALS AND METHODS: In the pre-treatment phase of the study all oocytes and embryos were cultured in G-III Series™ media. Embryos for transfer were chosen based on their Day 3 morphology. During the treatment phase embryos were equally divided between two separate media systems, either Vitrolife's G-III system or Irvine Scientific's Early Cleavage Media (ECM™) for Day 0-3 and MultiBlast™ Media from Day 3-6. Each oocyte or embryo was cultured individually in 20 µl drops of media under an oil overlay. Oocytes were placed into one of the two media on Day 0 and remained in that manufacturer's media through Day 6. Oocytes and embryos were cultured in CO2 in air through Day 3 and under low oxygen from Day 3 to 6. Embryo development was assessed at 16-20 hrs, 25-27 hrs, 40-43 hrs and 66-68 hrs post-insemination and scored according to the method of Fisch, et al. 2001. The maximum number of points an embryo could achieve was 100. Embryos were selected for transfer on Day 3 based on the total point score of each embryo and the leading embryos chosen, irrespective of media source. Any embryo not transferred or frozen by Day 3 was kept in culture through Day 6. Average patient age was 35.7 and the average number of embryos transferred was 2.4 in women under 40 and 4.8 for women over 40.

RESULTS: Simultaneous culture of oocytes and embryos in two separate sequential media series as opposed to culture in only one series of media improved clinical pregnancy rates (PR) per retrieval (presence of a gestational sac plus fetal heart tones) for women under 40 from 43% for Jan-Oct 2004 (N=173) to 67% for Nov 2004-present (N= 76; p=0.0024), and implantation rates (IR) from 25% to 38% (p = 0.002). However, simultaneous culture in two media did not affect PR or IR in women >39 as compared to culture in a single series (N= 19 vs 8; PR = 13% vs 30% and IM = 9% vs 3%). Fertilization rates did not differ between the two media (66% for both). Leading embryos arose with similar frequency in both media but the media that proved "best" for each individual patient differed from patient to patient. Transferring one leading embryo from each media series lead to the best pregnancy rates (87%) compared to the transfer of all embryos from only one source (60% for either).

CONCLUSION: Using two sequential media systems for the simultaneous culture of oocytes and embryos, as well as applying Fisch's scoring system, improved pregnancy outcomes for women under 40, but not for women 40 and older.

SUPPORT: ECM and MultiBlast media were provided at no charge from November 2004 through March 2005 by Irvine Scientific.

II BACKGROUND

- The traditional approach to embryo culture has been to employ the use of one, or possibly a single series of sequential, media system.
- However, human populations are not homogeneous. Furthermore, as is well known to laboratory scientists, human oocyte populations are also not homogeneous.
- Therefore, it is possible that there may not be one perfect media system, whether sequential or not, that will meet all the needs of every oocyte/embryo from every patient.
- An alternative may be to use more than one system.

III METHODS

- From January through October 2004 (Pre-treatment phase) all embryos were cultured in a single media series (G-III series media from Vitrolife)
- From November 2004 to the April 2005 (Treatment phase) oocytes were evenly divided between two separate manufacturer's media system (Early Cleavage Media and MultiBlast from Irvine Scientific and G-III series media from Vitrolife).
- Once allocated to a particular media system the oocyte and then the embryo remained in that media system throughout culture.
- The best embryos chosen for transfer on Day 3 regardless of media origination.
- Embryos were chosen for transfer based on their morphology according to Fisch et. al. 2001.
- All transfers, in all phases of the study, were done using Embryo Glue.

IV FINDINGS

Graduated Embryo Scoring System

Hours Post Insem.	Developmental Milestone	Points
16-18	Nucleoli aligned along pronuclear axis	20
25-27	Cleavage symmetrical and	30
	No fragmentation	30
	if <20%	25
	if >20%	0
40-42	Multinucleation	- all points
64-67	7-9 cell, even, 0% frag,	20
	8 cell, sltly uneven, <20%,	20
	or 7,9,10, symm.,<20% frags	10
Total possible		100

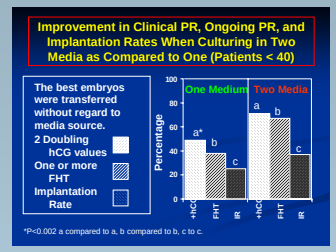
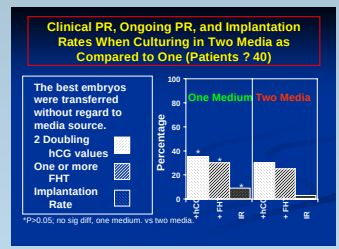


Figure 1.



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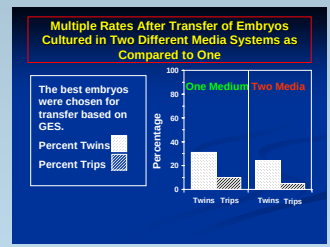

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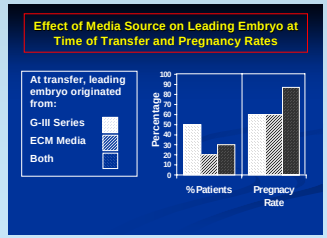

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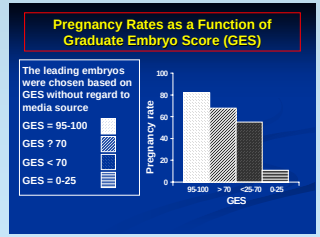

Figure 5.

V CONCLUSIONS

1. Pregnancy rates did not differ between the two media if transferred embryos were from one media.
2. Pregnancy rates improved in women under 40 if at least one embryo was transferred from both media systems.
3. Simultaneous culture in more than one media system did not improve pregnancy rates in women over 40.

[1]Fisch JD, et al. The Graduated Embryo Score (GES) predicts blastocyst formation and pregnancy rate from cleavage stage embryos. *Hum.Reprod.* 16:1970-1975, 2001.

[2]Fisch JD, et al. The graduated embryo score predicts the outcome of assisted reproductive technologies better than a single day 3 evaluation and achieves results associated with blastocyst transfer from day 3 embryo transfer. *Fertil. Steril.* 80; 1352-1358, 2003