Effects of transfer of embryos independently cultured in essential and sequential culture media on pregnancy rates in assisted reproduction cycles

Selmo Geber · Renata Bossi · Fernando Guimarães · Marcello Valle · Marcos Sampaio

Received: 25 March 2012 / Accepted: 13 July 2012 / Published online: 3 August 2012
© Springer Science+Business Media, LLC 2012

Abstract

Purpose Several culture media are available to be used in ART. However it is uncertain whether embryos would preferably benefit from one type of medium or the association of different media.

Methods We performed this study to evaluate the impact of simultaneous transfer of embryos independently cultured in two distinct culture media, on pregnancy outcome. A total of 722 couples who underwent infertility treatment were sequentially allocated into three groups: those who had half of the embryos individually cultured in MEM and the other half cultured in sequential media (MEM + Seq Group) ($n=243$); those who had all embryos cultured only in sequential medium (Seq Group) ($n=239$); and those who had all embryos cultured only in MEM (MEM Group) ($n=240$).

Results The pregnancy rate was higher in the MEM + Seq group (51.8 %) than the Seq group (36.7 %) ($p<0.001$). However the pregnancy rate observed in the MEM group was similar to the others (44.2 %). When a logistic regression test was applied it demonstrated that the number of transferred embryos did not interfere in the pregnancy rates.

Conclusions Our results suggests that offering different culture conditions for sibling embryos with subsequent transfer

of embryos that were kept in distinct culture media, might increase pregnancy rates in assisted reproduction cycles.

Keywords Culture media · Embryo development · Pregnancy rates

Introduction

One of the most important steps during assisted reproduction treatment is the adequate culture of embryos. Several culture media protocols have been formulated to this date, which are currently used in assisted reproduction centers worldwide [1–3]. The main purpose is to improve embryo development in vitro by mimicking their physiologic environment in the female reproductive tract, also regarding their biochemical, genetic and epigenetic developmental control.

The first culture media used in IVF cycles were minimum essential media (MEM) and were basically composed of salts, glucose and phenol red. The media were then supplemented with pyruvate and albumin [4, 5]. Other ingredients, such as taurine, EDTA, magnesium, citric acid and alanyl-glutamine were also added to the medium in order to enhance optimal culture conditions and improve embryo viability [6]. Later, sequential culture media were formulated and they presented differential composition according to the developmental stage of the embryo and its dynamic metabolic requirements [1, 7, 8]. In these culture media protocols, the first medium composition is based on the levels of carbohydrates present in the Fallopian tubes; the second medium is based on the levels of carbohydrates present in the uterus, and it also contains essential and non-essential amino acids, as well as vitamins, that enhance blastocyst formation and differentiation [8].

In spite of the current advances in the formulation of complex culture media, there is no consistent evidence that one protocol is substantially superior to the others,

Capsule Offering different culture conditions for sibling embryos with subsequent transfer of embryos that were kept in distinct culture media, might increase pregnancy rates in assisted reproduction cycles.

S. Geber · R. Bossi · F. Guimarães · M. Valle · M. Sampaio
Origen—Center for Reproductive Medicine,
Belo Horizonte, Minas Gerais, Brazil

S. Geber
Medical School of the Universidade Federal de Minas Gerais,
Belo Horizonte, Minas Gerais, Brazil

S. Geber (✉)
Av. Contorno 7747 Lourdes,
Belo Horizonte, MG CEP 30 110 120, Brazil
e-mail: selmogeber@origen.com.br

concerning in vitro embryo development and pregnancy rates. Moreover, the assumption that one specific ingredient would be harmful to the preimplantation embryo, regardless of its concentration and interactions in the medium, is still speculative [4, 9]. Different sequential culture protocols have been extensively tested for comparison purposes, in order to increase the ability to maintain in vitro embryo viability through the preimplantation period [10–13]. Nevertheless, it is uncertain whether embryos would preferably benefit from one type of medium in detriment of others or even if successful pregnancy rates would increase when combining different media composition, as distinct embryos may present unique metabolic needs.

Therefore, the objective of the present study is to evaluate whether the use of two distinct culture media (essential and sequential) in the same assisted reproduction treatment cycle affects pregnancy outcome, when at least two good quality embryos, one from each culture media, are simultaneously transferred to the uterus.

Materials and methods

This prospective study included a total of 722 couples who underwent infertility treatment through assisted reproduction technique from January 2007 to July 2008. The couples were sequentially allocated into three groups: those who had half of the embryos individually cultured in MEM and the other half cultured in sequential media (MEM + Seq Group) ($n=243$); those who had all embryos cultured only in sequential medium (Seq Group) ($n=239$); and those who had all embryos cultured only in MEM (MEM Group) ($n=240$). This study was approved by our institutional ethics committee according to the code of ethics of the *Conselho Federal de Medicina* (Brazilian National Medical Council).

Ovulation induction

All patients had the same protocols for ovulation induction, using the same hormones and the same criteria for dose tailoring. Only patients undergoing first or second cycles were included. Ovulation induction was performed using long protocol or antagonist protocol.

For long protocol, treatment started with subcutaneous administration of 3.75 mg of gonadotropin releasing hormone (GnRH) agonist (Lectrum, Sandoz, Brazil) for suppression of pituitary function [14]. To confirm down regulation, serum oestradiol concentrations and vaginal ultrasound were performed ~10 days later. If the oestradiol concentration was <30 pg/ml and the ultrasound showed an endometrial thickness of <3 mm, patients were considered ready to start ovulation induction. After confirmation of suppression, patients underwent ovulation induction with

daily recombinant FSH (rFSH; Gonalf, Serono, Brazil) subcutaneous injections. The starting dose was defined according to the patient's age, i.e. 225 IU for <35 years old, 300 IU for 35–37 years old and 375 IU for >37 years old. Antagonist protocol was indicated for patients >40 years old or poor responders, treatment started on day 2 of menstrual cycle using rFSH. When follicles reached 14 mm, patients started GnRH antagonist (Cetrotide, Serono, Brazil) treatment associated to rFSH. The dose of rFSH was tailored according to the ovarian response measured by oestradiol serum concentrations and follicular growth monitored by vaginal ultrasound. Oocyte maturation was induced with recombinant human chorionic gonadotrophin (250 μ g, hCG; Ovidrel, Serono, Brazil) when at least two follicles reached a mean size of 17 mm with concordant oestradiol levels (~ 200 pg/ml).

In vitro fertilization, embryo culture and transfer

Oocyte retrieval was performed ~34 h after hCG injection by vaginal ultrasound guided aspiration. Oocytes were transferred to 1 ml tubes (Falcon-BD, USA) containing culture medium. In the MEM + Seq group, oocytes were allocated in either MEM or sequential medium. In the MEM group and Seq group, all oocytes were allocated in the respective culture medium. After 5 h all metaphase II oocytes were inseminated by ICSI, as previously reported [15].

On the following day, i.e. 17–19 h later (day 1), the oocytes were checked for normal fertilization by the presence of two pronuclei. The embryos were cultured at 37 °C in a Petri dish (Falcon-BD, USA) under mineral oil (Sigma), under a gas phase of 6 % CO₂ and were checked daily for classification based on standard morphological parameters until transfer [16]. The MEM used was Earle's Balanced Salt Solution (EBSS—Sigma, USA), supplemented with 10 % substitute synthetic serum (SSS) and 0.47 mM pyruvate; the sequential culture media used was composed by two culture media—G-IVF (Vitrolife, Sweden) for gamete manipulation and G1 (Vitrolife) for embryo culture up to day 3. All embryos were transferred in G2 medium (Vitrolife). These culture media were supplemented with human serum albumin (HSA—Vitrolife).

On day 2 or 3 after oocyte retrieval, the embryos were examined and a maximum of four were selected for embryo transfer, depending on the patient's age and embryo quality. Patients <30 years received 2 embryos, ≥ 30 and ≤ 35 received 2 for first attempt and 3 for second attempt, >35 and ≤ 40 received 3 for the first attempt and 4 for the second attempt, and those ≥ 40 years old received 4 embryos when available. Patients of the MEM + Seq group received at least 1 embryo that was cultured in each culture media. All embryo transfers were performed using a soft transfer catheter (Sydney IVF—Cook—Australia) under transabdominal ultrasonographic guidance. In all cases the luteal phase was supported with vaginal progesterone (Crinone—Serono, Brazil) starting on

the evening of day 1 after oocyte retrieval [17]. Embryos not selected for transfer were cryopreserved on the same day. Serum β -hCG was measured 14 days after oocyte retrieval. Confirmation of pregnancy was made by vaginal ultrasonography at 2 and 4 weeks, when a fetal heart beat was observed. All pregnancies were followed at least for 20 weeks. The primary outcome measurement considered for analysis was the pregnancy rate, defined as the percentage of pregnancies with a fetal heart beat per embryo transfer.

Statistical analysis

Statistical analysis was performed using Chi-squared test associated to the Bonferroni correction method to compare pregnancy rates among the three groups. Analysis of variance was used to compare the number of oocytes, good quality embryos and transferred embryos. Duncan's multiple range test was applied when significance was observed. A model of logistic regression evaluated the interference of these factors on pregnancy rates. A sample size of 300 cycles in each group was estimated in order to obtain an increase of 10 % on pregnancy rates with $\alpha=0.05$ and $\beta=0.20$. Difference was considered significant when $P<0.05$.

Results

The mean age of the patients, the BMI, the cause of infertility, the duration of ovulation induction and the total rFSH dose was similar in the three groups (Table 1). The number of patients undergoing ovulation induction with antagonists was similar in all three groups.

The mean number of Metaphase II oocytes retrieved in the MEM + Seq group and in the seq group was similar and significantly higher than the observed in the MEM group ($p<0.001$). The number of patients that received day 3 embryo transfer was similar in the three groups (81, 80 and 80, respectively for MEM + Seq, Seq and MEM group). The mean number of good quality embryos (grade I/II) in the MEM + Seq group and in the MEM group was similar and significantly higher than the observed in the Seq group ($p<0.001$). The mean number of transferred embryos in Seq

group and in the MEM group was similar and both were significantly lower than the observed in the MEM + Seq group ($p<0.001$). The pregnancy rate was higher in the MEM + Seq group than the Seq group ($p<0.001$). However the pregnancy rate observed in the MEM group was similar to the others (Table 2). When a logistic regression test was applied it demonstrated that the number of transferred embryos did not interfere in the pregnancy rates.

Discussion

The recent advances achieved in the culture of preimplantation embryos in vitro and the development of new culture media has led to improvements in the culture protocols used in assisted reproduction [3, 4]. Two different culture protocols have been used, simple protocols containing essential media and sequential protocols. The present study evaluated the effects of culturing embryos from the same assisted reproduction treatment cycle in two different culture media and compared the results to cycles in which embryos were culture in one culture media.

As patient characteristics were similar in all groups we consider that the main difference in the results was based on the effect of the different culture media used for embryo culture in the different groups. Also, we did not compare embryo transfers performed on day 2 to those performed on day 3, as the results observed in our center are historically the same for both situations, in accordance to the described by others [18, 19]. Our results showed a higher pregnancy rate in the group of patients that had at least one transferred embryo that had been cultured in each culture media. The difference is probably due to the exposure of the transferred embryos to different metabolic environment. Also, these results suggest that distinct embryos from the same couple may present unique physiological needs during preimplantation development, an aspect that highlights the importance of providing different conditions or metabolic choices in the microenvironment in which embryos are maintained until transfer to the uterus. This is in agreement with Pomeroy et al. [20] that described a higher pregnancy rate for embryo transfers performed with embryos that were culture in both

Table 1 Patient characteristics and assisted reproduction treatment clinical outcomes according to type of culture media used for embryo culture

Parameter	MEM + Seq group	Seq group	MEM group	P
No. of patients	243	239	240	
Age	33.9 $\pm$ 4.8 (24–44)	34.1 $\pm$ 5.2 (22–45)	34.9 $\pm$ 5.4 (21–45)	NS
Body mass index	27.88 $\pm$ 5.35 (19.7–40.2)	27.26 $\pm$ 5.34 (19.9–39.9)	27.55 $\pm$ 5.66 (20.2–39.5)	NS
Duration of ovulation induction (days)	11.3 $\pm$ 0.7	11.1 $\pm$ 0.7	11.2 $\pm$ 0.8	NS
Total rFSH dose (IU)	2857.5 $\pm$ 171.2	2835 $\pm$ 186.4	2880 $\pm$ 150.8	NS

Results are mean $\pm$ SD (range)

Table 2 Assisted reproduction treatment laboratorial outcomes and pregnancy rates according to type of culture media used for embryo culture

Parameter	MEM + Seq group	Seq group	MEM group	<i>p</i>
MII oocytes retrieved ^a	10.7±5.1 (1–36)	9.8±6.6 (1–33)	7.4±5.2 (1–40)	<0.001
Good quality embryos ^b	4.5±2.8 (0–14)	3.9±2.9 (0–14)	4.6±2.9 (0–15)	<0.001
Embryos transferred ^c	3.9±1 (2–4)	3.2±1.1 (1–4)	3.2±1.1 (1–4)	<0.001
Pregnancy rate	51.9 %	36.7 %	44.2 %	<0.001

Results are mean ± SD (range)

^a MEM + Seq = Seq > MEM

^b MEM + Seq = MEM > Seq

^c Seq = MEM < MEM + Seq

^d MEM = MEM + Seq > Seq = MEM

sequential and nonsequential culture medium, when analyzing 144 patients. Also, Wirleitner et al. [21] retrospectively studied 286 cycles and described that the transfer of two embryos where one embryo was cultured in a different culture medium from the other embryo, resulted in a significantly higher rate of twin pregnancies.

The essential medium is composed of salts, glucose and was supplemented with pyruvate. Nitrogen source is provided by supplementation with substitute synthetic serum (SSS). The sequential media, however, provided more complex energy pathways, which included pyruvate, lactate and glucose, as well as amino acids as nitrogen source. In addition, it was supplemented with human serum albumin. The addition of nitrogen sources for embryo culture is not a consensus as some authors [22] suggests it is not fundamental for the adequate embryo development. Others [23] support the inclusion of all 20 amino acids to the culture medium at physiological levels, to let the embryos choose which to consume. Also, some changes in the pattern of amino acid turnover throughout human preimplantation embryo development in vitro have been described [24].

Concerning energy pathways, it has been demonstrated that from zygote to eight-cell stage, pyruvate is the main source used by the embryo to produce ATP [25–27]. Also, its uptake decreases after eight-cell stage while an increase on glucose uptake is observed [27]. Although some authors [28] suggested that the presence of glucose might compromise the viability of embryo development in vitro, our results corroborate the findings of Barak et al. [29] and Macklon et al. [30], that demonstrated that glucose does not necessarily inhibit early embryo development, as both culture media used in the our study had glucose in its composition, and good quality embryos were similarly obtained in all groups.

In summary, our results suggests that offering different culture conditions for sibling embryos with subsequent transfer of embryos that were kept in distinct culture media, might increase pregnancy rates in assisted reproduction

cycles. Further randomized and controlled studies are necessary to confirm our findings.

References

1. Jones GM, Trounson AO, Gardner DK, et al. Evolution of a culture protocol for successful blastocyst development and pregnancy. *Hum Reprod.* 1998;13:169–77.
2. Perin PM, Maluf M, Nicolosi Foltran Januário DA, et al. Comparison of the efficacy of two commercially available media for culturing one-cell embryos in the in vitro fertilization mouse model. *Fertil Steril.* 2008;90:1503–10.
3. Summers MC, Biggers JD. Chemically defined media and the culture of mammalian preimplantation embryos; historical perspective and current issues. *Hum Reprod Update.* 2003;9:557–82.
4. Biggers JD, Summers MC. Choosing a culture medium: making informed choices. *Fertil Steril.* 2008;90:473–83.
5. Biggers JD, Whittingham DG, Donahue RP. The pattern of energy metabolism in the mouse oocyte and zygote. *Proc Natl Acad Sci USA.* 1967;58:560–7.
6. Cooke S, Quinn P, Kime L, et al. Improvement in early human embryo development using new formulation sequential stage-specific culture media. *Fertil Steril.* 2002;78:1254–60.
7. Gardner DK. Development of serum-free media for the culture and transfer of human blastocysts. *Hum Reprod.* 1998;13 Suppl 4:218–25.
8. Gardner DK, Lane M. Culture of viable human blastocysts in defined sequential serum-free media. *Hum Reprod.* 1998;13 Suppl 3:148–59.
9. Pool TB. An update on embryo culture for human assisted reproductive technology: media, performance, and safety. *Semin Reprod Med.* 2005;23:309–18.
10. Balaban B, Urman B. Comparison of two sequential media for culturing cleavage-stage embryos and blastocysts: embryo characteristics and clinical outcome. *Reprod Biomed Online.* 2005;10:485–91.
11. Gardner DK. Dissection of culture media for embryos: the most important and less important components and characteristics. *Reprod Fertil Dev.* 2008;20:9–18.
12. Hambiliki F, Sandell P, Yaldir F, et al. A prospective randomized sibling-oocyte study of two media systems for culturing cleavage-stage embryos-impact on fertilization rate. *J Assist Reprod Genet.* 2011;28:335–41.
13. Van Langendonck A, Demyelle D, Wyns C, et al. 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;76:1023–31.

14. Geber S, Sales L, Sampaio M. Comparison between a single dose of goserelin (depot) and multiple daily doses of leuprolide acetate for pituitary suppression in IVF treatment: a clinical endocrinological study of the ovarian response. *J Assist Reprod Genet.* 2002;19:313–8.
15. Geber S, Sales L, Sampaio M. Laboratory techniques for human embryos. *Reprod Biomed Online.* 2002;5:211–8.
16. Veeck L. The morphological assesment of human oocytes and early concepti. In: Keel BA, Webster RW, editors. *Handbook of laboratory diagnosis and treatment infertility.* Boca Raton, FL: CR Press; 1990. p. 353–69.
17. Geber S, Moreira AC, de Paula SO, et al. Comparison between two forms of vaginally administered progesterone for luteal phase support in assisted reproduction cycles. *Reprod Biomed Online.* 2007;14:155–8.
18. Ertzeid G, Dale PO, Tanbo T, et al. Clinical outcome of day 2 versus day 3 embryo transfer using serum-free culture media: a prospective randomized study. *J Assist Reprod Genet.* 1999;16:529–34.
19. Shahine LK, Milki AA, Westphal LM, et al. Day 2 versus day 3 embryo transfer in poor responders: a prospective randomized trial. *Fertil Steril.* 2011;95:330–2.
20. Pomeroy KO, Foley S, Faber B, et al. A comparison of sequential medium with non-sequential medium: do some patients' embryos culture better in one than in the other? *Reprod Fertil Dev.* 2008;21:162–3.
21. Wirleitner B, Vanderzwalmen P, Stecher A, et al. Individual demands of human embryos on IVF culture medium: influence on blastocyst development and pregnancy outcome. *Reprod Biomed Online.* 2010;21:776–82.
22. Caro CM, Trounson A. The effect of protein on preimplantation mouse embryo development in vitro. *J In Vitro Embryo Transfer.* 1984;1:183–7.
23. Leese HJ, Sturmey RG, Baumann CG, et al. Embryo viability and metabolism: obeying the quiet rules. *Hum Reprod.* 2007;22:3047–50.
24. Brison DR, Houghton FD, Falconer D, et al. Identification of viable embryos in IVF by non-invasive measurement of amino acid turnover. *Hum Reprod.* 2004;19:2319–24.
25. Conaghan J, Handyside AH, Winston RML, et al. Effects of pyruvate and glucose on the development of human preimplantation embryos in vitro. *J Reprod Fertil.* 1993;99:87–95.
26. Hardy K, Hooper MAK, Handyside AH, et al. Non-invasive measurement of glucose and pyruvate uptake by individual human oocytes and preimplantation embryos. *Hum Reprod.* 1989;4:188–91.
27. Leese HJ, Conaghan J, Martin KL, et al. Early human embryo metabolism. *BioEssays.* 1993;15:259–64.
28. Devreker F, Englert Y. In vitro development and metabolism of the human embryo up to the blastocyst stage. *Eur J Obstet Gyn Reprod Biol.* 2000;92:51–6.
29. Barak Y, Goldman S, Gonen Y, et al. Does glucose affect fertilization, development and pregnancy rates of human in-vitro fertilized oocytes? *Hum Reprod.* 1998;13 Suppl 4:203–11.
30. Macklon NS, Pieters MHEC, Hassan MA, et al. A prospective randomized comparison of sequential versus monoculture systems for in-vitro human blastocyst development. *Hum Reprod.* 2002;17:2700–5.