Dynamics of human embryo development in two types of blastocyst media: a prospective trial on sibling oocytes

Dinamika razvoja človeških zarodkov v dveh vrstah gojišč za blastociste: prospektivna raziskava na sestrskih jajčnih celicah

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Ključne besede:
blastocista, gojišča, brazdanje zarodka, morfologija zarodka, implantacija

Key words:
blastocyst, culture media, embryo cleavage, embryo morphology, implantation

Citirajte kot/Cite as:
Zdrav Vestn 2011; 80: I-46–I-57

Prispelo: 7. dec. 2009,
Sprejeto: 30. sept. 2010

Abstract

Background: There is a lack of clinical trials on the success of embryo culture in different commercial culture systems in the programme of *in vitro* fertilization (IVF). In our prospective study, embryo development was compared in two sequential media.

Methods: The oocytes from each of 62 patients, treated by IVF/ICSI, were distributed into two groups for 5-day culture in the BlastAssist® System (Medicult, Denmark) (Group 1, n = 421) and in the GIII Series™ (Vitrolife, Sweden) (Group 2, n = 415) media. The dynamics of embryo development, implantation and pregnancy rates were compared between groups.

Results: The differences between Groups 1 and 2 were first noted on day 3 when GIII yielded more fast-cleaving (> 6 cells) nonfragmented embryos (31.3 % vs. 44.4 %, P < 0.01). On day 4 however, a larger number of compact embryos was found in Group 1 (42.2 % vs. 28.7 %, P < 0.005). The percentage of blastocysts or compact morulae on day 5 was the same in both groups (59.6 % vs. 55.6 %). But expanded blastocysts of optimal quality with oval inner-cell-mass developed more frequently in Group 1 (29.2 % vs. 17.7 %, P < 0.05), and therefore more transfers were performed exclusively with Group 1 embryos (28 vs. 17). Pregnancy and implantation rates were the same in both groups (53.6 % vs. 52.9 % and 34.7 % vs. 34.2 %, respectively).

Conclusions: Although the GIII Series™ yields more fast-cleaving embryos on day 3, embryo compaction (morula) and cavitation (blastocoel) start earlier in the BlastAssist® System media. BlastAssist® System therefore allows easier selection of embryos for transfer on day 5.

Izvleček

Izhodišče: V literaturi primanjkuje primerjalnih kliničnih raziskav o uspešnosti gojenja zarodkov v programu fertilizacije *in vitro* (IVF) v različnih komercialnih gojiščih. V naši prospektivni raziskavi smo primerjali razvoj zarodkov v dveh različnih sekvencijskih gojiščih.

Metode: Jajčne celice vsakega od 62 parov, ki so bili v postopku IVF/ICSI, smo razdelili v dve skupini. V prvi skupini (Skupina 1, n=421) smo zarodke gojili pet dni v gojiščih BlastAssist® System (Medicult, Denmark), v drugi skupini (Skupina 2, n=415) pa v gojiščih GIII Series™ (Vitrolife, Sweden). Med skupinama smo primerjali dinamiko embrionalnega razvoja, implantacijo in delež zanositev.

Rezultati: Razliko med skupinama 1 in 2 smo najprej opazili tretji dan inkubacije, ko se je v GIII gojišču razvilo več večceličnih (> 6 celic) nefragmentiranih zarodkov (31, 3 % oz. 44,4 %; P < 0,01). Četrti dan je bilo več kompaktnih morul zabeleženih v skupini 1 (42,2 % oz. 28,7 %; P < 0,005). Peti dan razvoja je bil delež blastocist in morul skupaj enak v obeh skupinah (59,6 % oz. 55,6 %), vendar pa se je v Skupini 1 razvilo več ekspandiranih blastocist optimalne kakovosti z ovalno notranjo celično maso (29,2 % oz. 17,7 %; P < 0,05), zato smo v maternico pogosteje prenesli zarodke prav iz skupine 1 (28 oz. 17). Stopnja zanositve in implantacije je bila med skupinama primerljiva (53,6 % oz. 52,9 % in 34,7 % oz. 34,2 %).

Zaključki: Čeprav so se do tretjega dneva starosti zarodki hitreje delili v gojiščih GIII Series™, sta se kompaktnost (morula) in kavitacija (blastocel) zarodka pojavila prej v gojiščih BlastAssist® System. BlastAssist® System je torej gojišče, ki omogoča boljšo selekcijo zarodkov za prenos v maternico na peti dan razvoja.

Introduction

The first reports on the use of synthetic media for mammalian embryos reach back as far as 50 years. At that time Whitten reported that mouse embryos could develop to blastocysts if cultured in a simple medium of only 9 components.[1] Since then intensive research has been going on to develop optimum chemically defined media, also suitable for human embryo culturing.[2] Most experiments were performed on animal models.

In various parts of the female reproductive tract, a precise analysis of the concentration of Na^+, K^+, Ca^{2+}, Cl^-, pyruvate, lactate and glucose was successfully carried out.[3,4] Assessment of the consumption of various energy substrates was also made possible. It was demonstrated in the human preimplantation embryo that the uptake of glucose relative to pyruvate increased after the 8-cell stage.[5-7] Among early and more than 3–day-old embryos different demands for essential and nonessential amino acids were also discovered.[8,9] Establishing metabolic differences between early embryos and blastocysts has lead to the development of sequential media for prolonged cultivation of embryos.[8-12] These media replaced the previous technically more demanding method of embryo co-culturing with other cells of the female reproductive tract.[13,14]

Biological supplements to the media, such as serum, were also abandoned. Instead, purer macromolecules, such as human serum albumins (HSA), are used, and in recent years these also are being replaced with safer and less varying components, such as recombinant albumins[15,16] or synthetic serum replacements.[17] In recent years, some other macromolecules, which enhance embryo development and implantation, e. g. EDTA[15] and hyaluronan,[18,19] have been added to enrich the media. Also, the toxicity of the medium occurring due to the degradation of amino acids is decreased by substituting the heat-sensitive glutamine with the more stable alanyl-glutamine.[20]

Today the majority of IVF media are commercial products. With the commercialization of the media, however, the concealing of data on their composition is more and more evident (see review from Biggers).[21] Randomized prospective preclinical and clinical studies on human embryos are also lacking, although the use of unsuitable media involves the potential risk of malformation occurrence.[22]

Comparisons of the effect of different media, based on following the development of sibling oocytes, are particularly rare. None are to be found for the testing of embryonic development of human embryos to the blastocyst stage or for clinical results after using the BlastAssist® System (Medicult, Jyllinge, Denmark) and G III Series™ (Vitrolife, Gothenburg, Sweden).

Material and Methods

Study design

The study was performed in the period from January to March 2006. Sixty-two consecutive hormonally stimulated IVF/intracytoplasmic sperm injection (ICSI) cycles in which more than 5 oocytes were obtained after oocyte pick-up were included in the prospective study in which two commercial sequential media were tested by sibling oocytes. From oocyte retrieval to day 5 of cultivation, the first group of oocytes (n = 421) was cultured in BlastAssist® sequential media, and the second group of oocytes (n=415) in GIII Series™ sequential media.

The study was focused on the quality and dynamics of embryo development in both media and the implantation capacity of embryos conceived by means of either of both culture systems.

The complete assortment of media by Medicult or Vitrolife was also used separately for each group–for the preparation of sperm and for micromanipulation, and including paraffin oil–strictly following the instructions of both manufacturers. Before this study, our lab already had experience with using media produced by both manufacturers. Nevertheless, prior to the beginning of the study, experts from both firms paid separate visits to our laboratory, following all procedures in the handling of media and embryos. The authors of the present

paper derive no financial benefit from the manufacturers of the applied media, which were all purchased regularly.

Patients

The patients were unselected regarding age, infertility indications, number of previous IVF/ICSI attempts, dose of gonadotrophins required for ovulation stimulation, sperm parameters or method of insemination. The mean age of female patients was 31.9 ± 4.6. The indication for infertility treatment by assisted reproduction techniques (ART) was tubal factor in 29 %, endometriosis in 11.3 %, uterine factor in 6.5 %, male factor in 66.1 % and idiopathic infertility in 6.5 %. The patients were treated with an average 26.5 ± 7.7 (14 to 48) ampoules of gonadotrophins and the duration of stimulation was 10.3 ± 1.6 (7 to 15) days. After oocyte pick-up 13.6 ± 6.2 (6 to 37) oocytes were obtained. In 22.6 % (14/62) of cycles the classic IVF was used as the method of oocyte insemination while in 77.4 % (48/62) of cycles the oocytes were inseminated by ICSI. In 4.8 % (3/62) of cases ICSI was performed by using testicular sperm.

Ovarian stimulation

The women were synchronized with oral contraceptives, starting the long stimulation protocol with a daily application of triptorelin (Decapeptyl 0.1mg) or a single application of depot GnRHa goserelin (Zoladex 3.6 mg, Zeneca, Cheshire, UK). Pure FSH (Metrodin-HP, Serono, Geneve, Switzerland) or recombinant FSH (Gonal F, Serono, Geneve, Switzerland) was applied for controlled ovarian hyperstimulation. The daily FSH dose was adjusted to ovarian response to stimulation. On the day when folliculometry revealed that the mean diameter of the dominant follicle had reached 18 mm, the time for hCG injection was set.

All patients had hCG (Profasi, Serono, Auborne, Switzerland) applied subcutaneously in doses of 10,000 IU by self-administration. Follicular puncture followed 36–37 hours later.

Oocyte recovery

Cumulus-oocyte complexes from the follicular aspirate of each individual patient were chosen randomly for cultivation either in Medicult or *in Vitro*life media according to the quasi-randomisation principle »one here–one there«. In the Medicult group the oocytes were collected in Flushing Medium, and in the Vitrolife group in G-MOPS™ PLUS medium. Cumulus-oocyte complexes were incubated in oocyte collecting media for half an hour maximum.

Cumulus oocyte complexes designed for IVF were transferred to 0.5 ml BlastAssist® M1 medium or G-FERT™ PLUS in 4-well dishes (Nunclon, Roskilde, Denmark).

Sperm preparation and insemination

One half of the ejaculate was prepared with the Sperm Preparation Medium (Medicult) and the other half with G-SPERM™ PLUS (Vitrolife). A small amount (1 ml) of semen sample was diluted by culture medium at a ratio 1:3. The sample was centrifuged in a 5 ml tube for 10 minutes at 300g. Fresh Sperm Preparation Medium or G-FERT™ PLUS (Vitrolife) was poured on the sediment and the sample was incubated for 30–45 minutes. The fraction of motile sperm from the upper part of overlaid media was isolated and assessed for concentration. The sperm suspension was used for IVF: if it contained at least 6 million motile sperm/ml, if the native ejaculate contained over 20 million sperm with at least 40 % motility and if there was no total fertilization failure in previous IVF cycles. Otherwise, the sample was used for ICSI. In the IVF procedure the oocytes cultured in 0.7 ml of culture media were inseminated with 150,000 to 200,000 motile sperm.

The cumulus was removed from the oocytes for ICSI using SynVitro®Hyadase (Medicult) or HYASE™ (Vitrolife). The ICSI procedure was carried out in BlastAssist® M1 medium (Medicult) or G-OOCYTE™ medium with HSA™ (Vitrolife) added. The sperm for ICSI were immobilized in PVP Medium (Medicult) or in ICSI™ (Vitrolife). After the

ICSI procedure the oocytes were transferred to a drop of BlastAssist® M1 medium or G-1™ v3PLUS medium in 4-well dishes (Nunclon, Roskilde, Denmark).

The oocytes were evaluated for the presence of pronuclei 18–20 hours post insemination.

Embryo culture

For embryo culture the atmosphere with 6 % of CO_2 and 5 % of O_2 was used, because the beneficial effect of reduced oxygen concentration on embryo development has been proved.[23,24] All media were prepared the day before and were preincubated in the same atmosphere, with the exception of G-MOPS™ PLUS and G-OOCYTE™ media, which were only heated. The concentration of gasses in all incubators was monitored daily by an external unit. For embryo culture, only dishes (Nunclon, Roskilde, Denmark and Beckton Dickinson Labware, Franklin Lakes, NJ) tested for the presence of embryotoxins by mouse embryo assay were used. Embryos were cultured in groups in small drops of 60μl of medium, covered by liquid paraffin (Medicult) or Ovoil (Vitrolife) until day 5 in an atmosphere gassed with 6 % of CO_2 and 5 % of O_2.

The embryos were cultured in sequential media whose composition is shown in Table 1. The media were replaced daily with fresh ones.

All zygotes on day 1 were transferred to a 60μl drop of fresh BlastAssist® M1 medium or G-1™ v3PLUS medium in 4-well dishes. On day 2 the embryos were evaluated for morphology and grouped by morphology criteria.

On day 3 the embryos were transferred to the BlastAssist® M2 medium or G-2™ v3PLUS medium and evaluated again. The media were substituted with fresh ones on day 4.

Embryo grading

Embryo quality was evaluated on day 2 (44–48 hours post insemination) and on day 3 (68–72 hours post insemination) with grades from GI to GIV, depending on embryo fragmentation (GI: up to 10 % of fragments; GII: 10–20 % of fragments; GIII: 20–50 % of fragments; GIV: more than 50 % of fragments). For reasons of statistical analysis, GI and GII embryos were joined into one group (nonfragmented embryos) and GIII and GIV into another (fragmented embryos). Also the number of blastomeres was evaluated on day 2 and 3.

The embryos that reached the compact stage with more than 10 cells on day 4 or 5, were considered a compact morula.

Early blastocysts were defined as embryos in which the blastocoele filled less than half of the embryo.

Optimal blastocysts on day 5 had an expanded blastocoele, cohesive and multicellular trophectoderm and oval and compact inner cell mass.

Blastocyst transfer and pregnancy assessment

On day 5 (116–120 hours post insemination) one or two of the best blastocysts were selected for transfer, either from one or from both culture systems. The embryos for transfer were selected according to our previously described blastocyst grading system,[25] which considers different implantation ability of 8 morphological types of blastocysts and morulae on day 5. The blastocysts were transferred to EmbryoGlue™ (Vitrolife) for 10 minutes before transfer.

A catheter (Labotect, Göttingen, Germany) was loaded with 20 μl of medium containing embryos from under paraffin oil.

Evaluation of pregnancy and implantation

The pregnancy was tested 14 days after retrieval of oocytes by quantitative determination of beta hCG in the serum. Ultrasonographic evaluation of the number of gestational sacs was done 7 days after positive serum beta hCG (> 15 IU). Evaluation of cardiac activity was done 7 days later.

Statistical analysis

The data were stratified according to the type of culture media (BlastAssist® System

Table 1: Composition of sequential media M1/M2 BlastAssist® System (Medicult) and G-1™ v3 PLUS / G-2™ v3 PLUS (Vitrolife).

Components	BlastAssist® M1	BlastAssist® M2	G-1™ v3 PLUS	G-2™ v3 PLUS
Alanine	P	P	P	P
Alanyl-glutamine	-	-	P	P
Arginine	-	P	-	P
Asparagine	P	P	P	P
Aspartate	P	P	P	P
Calcium chloride	-	-	P	P
Calcium lactate	P	P	-	-
Calcium pantothenate	-	-	-	P
Cystein	-	P	-	-
Cystine	-	-	-	P
EDTA	P	P	P	-
Glucose	P	P	P	P
Glutamate	P	P	P	P
Glutamine	P	P	-	-
Glycine	P	P	P	P
Histidine	-	P	-	P
HEPES	P	-	-	-
Human Serum Albumin (HSA)	P	P	P	P
Hyaluronan	-	-	P	P
Isoleucine	-	P	-	P
Leucine	-	P	-	P
Lysine	-	P	-	P
Magnesium sulphate	P	P	P	P
Methionine	-	P	-	P
Penicillin G	P	P	P	P
Phenol Red	P	P	-	-
Phenylalanine	-	P	-	P
Potassium chloride	-	-	P	P
Potassium sulphate	P	P	-	-
Proline	P	P	P	P
Pyridoxine	-	-	-	P

Riboflavine	-	-	-	P
Serine	P	P	P	P
Syntetic Serum Replacement (SSR)	P	P	-	-
Sodium bicarbonate	P	P	P	P
Sodium chloride	P	P	P	P
Sodium dihydrogen phosphate	-	-	P	P
Sodium lactate	-	-	P	P
Sodium phosphate	P	P	-	-
Sodium pyruvate	P	P	P	P
Streptomycin	P	P	-	-
Taurine	P	-	P	-
Thiamine	-	-	-	P
Threonine	-	P	-	P
Tryptophan	-	P	-	P
Tyrosine	-	P	-	P
Valine	-	P	-	P

P = presence of the component; however, its millimolar concentration is unknown.

or G III Series™). The groups were compared using the chi-square test. A P value ≤ 0.05 was considered statistically significant.

Results

There were no statistically significant differences between Groups 1 and 2 regarding the overall fertilization (54.9 % vs. 54.2 %), fertilization after IVF (55.2 % vs. 47.8 %) and fertilization after ICSI (71 % vs. 71.2 %) (Table 2).

Dynamics and quality of embryo development on day 2 were the same in both groups.

On day 3, a significantly larger number of fast-cleaving (> 6 cells) and fast-cleaving good embryos (> 6 cells, nonfragmented) developed in Group 2 as compared to Group 1 (33.5 % vs. 55.6 %, P < 0.0001 and 31.3 % vs. 44.4 %, P < 0.01, respectively).

On day 4 however, a larger number of compact embryos was found in Group 1 than in Group 2 (42.2 % vs. 28.7 %, P< 0.005). In the remaining embryos, more noncompact morulae (≥ 10 cells) were present in Group 2 than in Group 1 (10.9 % vs. 25.6 %, P< 0.0001).

The proportion of blastocysts and compact morulae on day 5 was the same in both groups (59.6 % vs. 55.6 %), but expanded and optimal blastocysts with oval inner cell mass developed more frequently in Group 1 than in Group 2, (29.2 % vs. 17.7 %, P< 0.05). On day 5, fewer embryos from Group 1 were in the stages of early blastocyst and compact morula (11.7 % vs. 28.2 %, P < 0.005).

This resulted in slightly more embryos for transfer being chosen from Group 1 than from Group 2 (55 vs. 44), but the opposite was the case in blastocyst freezing (55 vs. 62).

Fifty-seven out of 62 study cycles were terminated by blastocyst transfer, resulting

in 30 ongoing pregnancies (52.6 % pregnancy rate per transfer). There were 5 cycles without embryo transfer: 3 total fertilization failure cycles, and 2 cycles with all embryos arrested.

More transfers with exclusively Group 1 embryos were performed due to faster cleavage and blastulation than transfers with only Group 2 embryos (28 vs.17) (Table 3). The clinical pregnancy rate was the same after those transfers in which the embryos derived exclusively from Group 1 (15/28, 53.6 %) or from Group 2 (9/17, 52.9 %). Also there was no statistically significant difference between delivery rates in both groups (14/28 (50 %) vs. 9/17 (52.9 %)). In 12 cases the embryos for transfer were chosen from both groups.

Elected single blastocyst transfer was performed in 16 patients: as many as 13 transfers of single blastocyst from BlastAssist and only 3 transfers of blastocysts from GIII media.

The implantation rate for the cycles in which the origin of transferred embryos was known (49 and 38 transferred blastocysts, respectively) was the same in both groups (34.7 % vs. 34.2 %) (Table 2).

Discussion

Both media belong among the first commercial media designed for prolonged culturing of human embryos to blastocysts. The GIII Series™ PLUS has a rich scientific background. Its development is based on numerous basic studies of human embryo metabolism and nutrient requirements. It is a partially synthetic medium with human albumins added, which simulates the chemical composition of various parts of the reproductive tract in animals and humans. The justified presence of most components is argued by studies on the development of animal and human embryos *in vitro* (see review by Gardner and Lane).[12] This medium is subject to continuous development. Today its fifth generation is already available. Its composition was transparent up to the second generation, showing millimolar concentrations of each medium component separately,[26] but for the third generation the manufacturer no longer reveals the concentrations.

The exact composition of BlastAssist® System media was never known. At least in the published literature there is no proof of medium development by extensive preclinical testing on animal material. The BlastAssist® System media are also partly synthetic media. Serum is substituted with the Synthetic Serum Replacement (SSR®), which however also contains human albumins.

It is difficult to find an explanation for the differences in embryonic development observed between both media if the composition of the media is not precisely known. Nevertheless, from the list of components in table 1 we can note certain differences between both media.

Instead of the heat-sensitive and unstable glutamine, the GIII Series™ medium–as opposed to the BlastAssist® System medium–contains its heat-stable peptide derivative, such as L-alanyl-L-glutamine. This substitution proved successful in mouse embryos, since it increases the percentage of blastocysts and the number of cells.[20] In this way, when heating the media up to 37 °C, the decomposition of glutamine and the release of ammonium into the medium – which occurs in media with glutamine presence – are prevented.[22,26] Namely, higher concentrations of ammonium are harmful to embryonic development in some mouse strains[27] while they have no effect on bovine embryos.[28] Due to the presence of a more stable glutamine form, the GIII Series™ media have a longer shelf-life than BlastAssist® System media.

To avoid the toxic effect of ammonium, the embryos were transferred daily to fresh medium, as is also advised by both manufacturers. Ammonium reduces blastocyst cell counts, decreases ICM and increases apoptosis.[27] Unfortunately, on account of the slower development of embryos in the GIII Series™ as compared to the BlastAssist® System media and the earlier termination of *in vitro* cultivation, we were not able to compare both groups with respect to the mentioned parameters.

A number of studies proved a better implantation rate if the medium contained

hyaluronan.[18,19,29] Despite the proven presence of glycosaminoglycans in the uterine, oviductal and follicular fluids,[30,31] it is not clear yet whether hyaluronan has a positive effect on implantation if the embryos are cultivated in medium with hyaluronan. It has also not been elucidated whether it is sufficient to merely expose the embryos to hyaluronan immediately prior to transfer to the uterus where its concentration is the highest.[32] Namely, both GIII Series™ media contain hyaluronan while the BlastAssist® System does not contain it. In our study, prior to transfer to the uterus, all blastocysts were transferred for 10 to 30 minutes to hyaluronan (EmbryoGlue™, Vitrolife).

The BlastAssist® M1 medium, where the oocytes were also kept during the ICSI procedure, contains HEPES (N-hydroxyethylpiperazine-N-ethanesulfonate) buffer. We found no reason for the presence of HEPES in BlastAssist® M1 cultivation medium and we believe it is not necessary, particularly so as its harmful effect on oocytes has been proven, above all if ICSI is carried out in a medium containing HEPES.[33] The manufacturer of BlastAssist® media recommends another medium for ICSI, but we have been using M1 successfully in our lab for several years. The G-1™ v3 PLUS and G-2™ v3 PLUS cultivation media contain no other buffer beside bicarbonate. In the GIII Series™ we carried out ICSI in G-OOCYTE™ medium, which contains MOPS buffer.

We also observed a difference in EDTA (ethylenediaminetetraacetic acid) presence. EDTA is an important chelator in numerous media, where it chelates the toxic heavy metal ions, which might be present in the media. It also inhibits premature utilization of glycolysis by cleavage stage embryos. Although EDTA stimulates the development of the cleavage stage embryos, its presence in the medium for postcompaction embryos significantly reduces subsequent blastocyst development and cell number, and inhibits the inner cell mass in the cow.[15] For this reason numerous authors advise against the use of this chelator in a second medium and that is why the G-2™ v3 PLUS medium does not contain it. In the BlastAssist® System it is present in M1 and M2 medium, although it is not known whether its concentration is any lower in M2 medium.

The significance of EDTA presence in both M1 and M2 BlastAssist® media has a different basis. The BlastAssist® System contains the patented Synthetic Serum Replacement, a synthetic metal ion buffer composed of a balanced mixture of iron and trace metals, but its actual composition is not known. We merely know that it contains no proteins, lipids or glycans except for recombinant insulin. Together with chelators, such as EDTA, they contribute to the stability of important metals, and EDTA artificially increases the purity of the culture medium by effectively removing reactive oxygen radicals or impurities such as heavy metals etc. from the media (Medicult media–manual). So, the stress tolerance of embryos in M2 BlastAssist®, which contains EDTA, could be higher than in G-2™ v3 PLUS medium, which does not contain it.

Despite the presence of SSR®, the BlastAssist® System–like the GIII Series™ PLUS media–also contains human serum albumins.

From the third generation G-2™ v3 PLUS medium the manufacturer removed some vitamins (choline cloride, folic acid, inositol and nicotinamide), which were still present in the second generation, preserving only pantothenate, pyridoxine, riboflavin and thiamine. Vitamins are not found on the list of BlastAssist® System components. The effect of vitamins on embryo development has not been sufficiently studied. Tsai and Gardner report that 5 microM nicotinamide inhibits mouse embryo development, reduces blastocyst cell number, implantation rate, viable pregnancies and fetal weight.[34] In another study they report that among the many vitamins pantothenate was the best for stimulation of blastocyst development in hamsters. Other vitamins did not impair the development.[35]

So the GIII Series™ and BlastAssist® System media differ in several important segments, which could affect embryo development. When using these two culture systems in our study, we observed some differences in embryonic development, which however were only expressed after three days of cultivation.

Table 2: Comparison of fertilization, embryo development, blastulation and implantation ability of blastocysts cultured in BlastAssist® System (Medicult) or GIIISeries™ (Vitrolife) media.

	BlastAssist® System	G III Series™	P[a]
Oocytes *(n)*	421	415	
Fertilization			
Normal fertilization	231 (54.9)	225 (54.2)	NS
after IVF	(55.2)	(47.8)	NS
after ICSI	(71)	(71.2)	NS
One pronucleate oocytes	8 (1.9)	10 (2.4)	NS
Three pronucleate oocytes	6 (1.4)	5 (1.2)	NS
Cleaved normal zygotes *(n)*	230	223	NS
Day 2 embryo quality			
> 2 cell, Grade I, II	139 (60.4)	128 (57.4)	NS
2 cell, Grade I, II	57 (24.8)	49 (22)	NS
> 2 cell, Grade III, IV	19 (8.3)	26 (11.7)	NS
2 cell, Grade III, IV	15 (6.5)	20 (9)	NS
Day 3 embryo quality			
> 6 cell, Grade I, II	72 (31.3)	99 (44.4)	< 0.01
≤ 6 cell, Grade I, II	115 (50)	73 (32.7)	< 0.0005
> 6 cell, Grade III, IV	5 (2.2)	21 (9.4)	< 0.001
≤ 6 cell, Grade III, IV	38 (16.5)	30 (13.5)	NS
Day 4 embryo quality			
Compact morula	97 (42.2)	64 (28.7)	< 0.005
≥ 10 cell embryo	25 (10.9)	57 (25.6)	< 0.0001
< 10 cell embryo	108 (46.9)	102 (45.7)	NS
Day 5 embryo quality			
Arrested embryos	93 (40.4)	99 (44.4)	NS
Blastocysts, compact morulae	137 (59.6)	124 (55.6)	NS
Expanded, oval ICM	40 (29.2)	22 (17.7)	< 0.05
Early blastocysts	16 (11.7)	35 (28.2)	< 0.005
Clinically used blastocysts	110	106	NS
Frozen blastocysts	55 (50)	62 (58.5)	NS
Transferred blastocysts	55 (50)	44 (41.5)	NS
Transferred blastocysts with known implantation outcome*	49/55	38/44	
Clinical implantations (gestational sac, heartbeats)	17/49 (34.7)	13/38 (34.2)	NS

Values in parentheses are percentages
ICM = Inner cell mass
NS = not significant
Grade I,II – embryos with up to 20 % of fragments
Grade III,IV – embryos with more than 20 % of fragments
[a] *chi-square test*
* *In 6 double blastocyst transfers (1 blastocyst from BlastAssist®, 1 blastocyst from GIII Series™), resulting pregnancies, a single gestational sac was observed and thus the origin of blastocysts was unknown*

Table 3: Clinical outcomes of cycles with transfers of blastocysts only from BlastAssist® System (Medicult) or GIIISeries™ (Vitrolife) media.

	BlastAssist® System	G III Series™	P[a]
Transfers of blastocysts from only Group 1 or 2*	28	17	
Transferred blastocysts	43	33	
Mean number of transferred blastocysts	1.5	1.9	
Optimal blastocysts	23/43 (53.5)	12/33 (36.4)	NS
Implantations (gestational sacs with heart beats)	16/43 (37.2)	12/33 (36.4)	NS
Clinical pregnancies	15/28 (53.6)	9/17 (52.9)	NS
Miscarriages	1	0	
Deliveries	14/28 (50)	9/17 (52.9)	NS

Values in parentheses are percentages.
[a] *chi-square test*
* *In 12 transfers the blastocysts were selected from both groups and were not included in this table.*

Until day 3 (68–72 hours post insemination), the embryos were cleaving more rapidly in G-1™ v3 PLUS than in M1 BlastAssist® medium (55.6 % vs. 33.5 % embryos with more than 6 blastomeres). Among these there were also more nonfragmented embryos in G-1™ v3 PLUS medium. In short-term cultivation with the GIII Series™ medium we thus get a larger number of embryos suitable for transfer or cryopresevation than with BlastAssist® M1 medium. These results are in accordance with those from studies comparing the BlastAssist® System and G1.2/G2.2 in randomized cycles, but not on sibling oocytes.[36]

The BlastAssist® System caused a faster compaction of blastomeres than the G III Series™. It is evident from our results that on day 4 (92–96 hours post insemination) only 28.7 % of embryos in the G III Series™reached the compact morula stage while in the BlastAssist® System there were as many as 42.2 %. This time difference in compaction can also mean only a few hours, since until day 5 (116–120 hours post insemination) the percentage of embryos reaching the blastocysts stage or at least the morula stage had equalized in both compared groups (59.6 % vs. 55.6 %).

Nevertheless, using the BlastAssist® System we succeeded in attaining a larger number of expanded blastocysts on day 5 and consequently also a larger number of blastocysts with an optimal oval ICM than in the G III Series™ media. Thus we may conclude that in the BlastAssist® System embryonic compaction and cavitation occurs earlier than in the GIII media. Although slower developing embryos are known to have a poorer implantation potential,[37] we cannot draw such conclusions since we know of 5-day embryonic stages in particular that their morphology and blastocoele expansion can change in a matter of hours.[25]

Owing to different dynamics of embryonic development, toward the end of cultivation we were not able to draw a comparison of the morphology of blastocyst inner cell mass between both systems. This would only be possible if the embryos reaching the compact morula or early blastocyst stage were cultivated for an additional day. Due to our previous bad experience with cultivation of embryos to day 6 in BlastAssist and with their freezing, the cultivation of embryos in our study was always terminated on day 5 (120–124 hours post insemination) regardless of the development of the blastocoele. Other studies frequently mention cultivation of embryos up to day 6, in BlastAssist® System[38] among others.

Be that as it may, the percentage of clinically used day 5 embryos for transfer and freezing together did not differ. But, due to the fixed duration of cultivation, we decided in several cases on the transfer of blastocysts from BlastAssist® System where blastocyst expansion occurred earlier.

In both groups, the percentage of implantations established in cycles with known origin of transferred embryos was also the same. Likewise, in a similar study by Zollner et al.[36] we also found that, despite the observed differences in embryonic development dynamics, the cultivation systems are comparable, although the BlastAssist® System allows easier selection of embryos for transfer on day 5.

An actual comparison of the effectiveness of both media would only be possible after assessing the cumulative pregnancy and implantation rate, under additional consideration of successful freezing and thawing of surplus day 5 embryos from both systems.

In both systems a separate comparison of embryonic development of morphologically poor and good embryos as well as of the clinical results in good- and poor-responder patients should be carried out. It would be interesting to establish the effectiveness of both systems in these specific cases and to find whether a prolonged cultivation of embryos in either one compromises embryonic development. So far, our studies with BlastAssist® System could not confirm any embryonic development compromise, not even in natural cycles[39] or in poor-responder patients.[40]

Summary

The comparison of two commercial blastocyst media showed differences in the dynamics of embryo development between the media. Pregnancy and implantation rates were the same in both groups. Although the GIIISeries™ yields more fast-cleaving embryos on day 3, embryo compaction and cavitation starts earlier in the BlastAssist® media. BlastAssist® therefore allows easier selection of embryos for transfer on day 5.

Acknowledgment

The authors wish to thank Nina Hojnik and Naca Herceg for their contribution in laboratory work.

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