Embryo culture media and IVF/ICSI success rates: a systematic review

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BACKGROUND: The media that are used to culture human preimplantation embryos are considered to be an important factor for the success rates of IVF/ICSI. Here, we present a systematic review of randomized controlled trials (RCTs) on the effect of culture media on IVF/ICSI success rates.

METHODS: RCTs published between January 1985 and July 2012 were eligible for inclusion. The primary outcome was live birth. Secondary outcomes were health of babies born, ongoing pregnancies, clinical pregnancies, miscarriages, multiple pregnancies, implantation rate, cryopreservation rate, embryo quality and fertilization rate. For those media that were evaluated in more than one comparison, an unconventional meta-analysis was performed by pooling the data of the media they were compared to.

RESULTS: Twenty-two RCTs were included that evaluated 31 different comparisons. Conventional meta-analysis was not possible for any of the outcomes as nearly all trials compared different culture media. Only four trials reported on live birth, and one of them reported a significant difference. Nine trials reported on ongoing and/or clinical pregnancy rates, of which four showed a significant difference. Pooling the data did not reveal a superior culture medium.

CONCLUSIONS: It is yet unknown what culture medium leads to the best success rates in IVF/ICSI. Given the potential importance of culture media for treatment outcome, rigorously designed RCTs are needed for currently available, as well as newly introduced culture media.

Key words: culture medium / IVF/ICSI / live birth / randomized controlled trial / meta-analysis

Introduction

Subfertility is of major clinical, social and economical concern. The most frequently used interventions are in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Despite their frequent use, the two largest data collections report a delivery rate per started cycle of only 18.4 and 25.2%, respectively (SART, 2009; de Mouzon et al., 2010). For a successful pregnancy to occur, good quality preimplantation embryos are essential. Several studies have suggested that culture media have an impact on the quality of embryos generated in IVF/ICSI cycles thereby influencing implantation and pregnancy rates.
from 1985 to 2012 were manually searched. Authors of included studies were contacted and if there was no response the paper was excluded.

The primary outcome of this review was live birth rate presented per woman randomized. Other outcomes were: health of the babies born (defined by the presence of congenital anomalies), birthweight, ongoing pregnancy rate (number of viable gestations with positive fetal heart beat per randomized woman), clinical pregnancy rate (number of clinical pregnancies demonstrated by the presence of a gestational sac on ultrasound scan per randomized woman), miscarriage rate (number of failed pregnancies up to 22 weeks of gestation per randomized woman) and multiple-pregnancy rate (number of multiple pregnancies per randomized woman). Embryo outcomes were: fertilization rate (number of oocytes fertilized per oocytes retrieved), number of top quality embryos at Day 3 per number of embryos, cryopreservation rate (number of embryos cryopreserved per randomized woman) and implantation rate (number of fetal sacs detected by ultrasound per number of transferred embryos).

Our analysis was on an intention to treat basis, hence studies not reporting on exact numbers of women per group were excluded from the analysis. To investigate whether any potential effect of the culture media on IVF/ICSI success rates depended on the day of embryo transfer, we subdivided the data according to the time of embryo transfer (Day 2–3 or Day 4–6). We anticipated that conventional meta-analysis might not be possible. To be able to recognize the potential superiority of one medium over several others, an unconventional hypothesis-generating meta-analysis on pregnancy rate per woman was done for media that were involved in multiple comparisons. For this meta-analysis, we used the random effect analysis model and the Mantel–Haenszel statistical method. As a criterion for heterogeneity among the studies, the $I^2$ was calculated for every comparison. The control group consisted of the pooled data of the combined comparison media. Data on clinical pregnancies, which were reported in the majority of the studies, were pooled and if these were not available, then the data from ongoing pregnancies were used. No subgroup analysis was performed and data from early and late embryo transfers were pooled together.

## Results

### Characteristics of included studies

Five hundred and sixty-six potentially relevant abstracts were identified by our search strategy. After reading the abstracts, 461 abstracts were excluded because they were comparing early versus late embryo transfer, because they were comparing media supplementation or because they were studying different species. Of the remaining 105 abstracts that appeared to meet the inclusion criteria, the full papers were retrieved. Another 65 papers were excluded after in-depth assessment of presented data. Reasons for exclusion were: the trial was not relevant to the topic under study (25), the trial reported incomplete data and no response was received after contacting the corresponding author (18), the trial was not an RCT (14), the trial was a republication of a previous trial (7) and the trial appeared to be a review (1) (Fig. 1 and Supplementary data, Table SII). Further, 18 studies were excluded because they were published as abstracts in

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**Methods**

Computerized searches were conducted using MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), the National Research Register, the Medical Research Council’s Clinical Trials Register and the NHS Centre for Reviews and Dissemination databases using the following medical subject headings and text words: (Keywords CONTAINS ‘IVF’ or ‘in vitro fertilization’ or ‘in-vitro fertilisation’ or ‘ICSI’ or ‘intracytoplasmic sperm injection’ or ‘Embryo’ or ‘ET’ or ‘Embryo Transfer’ or ‘in-vitro fertilization’ or Title CONTAINS ‘IVF’ or ‘in vitro fertilization’ or ‘in-vitro fertilisation’ or ‘ICSI’ or ‘intracytoplasmic sperm injection’ or ‘Embryo’ or ‘ET’ or ‘Embryo Transfer’ or ‘in-vitro fertilization’). AND (Keywords CONTAINS ‘embryo culture’ or ‘embryo culture media’ or ‘Culture-Media’ or ‘culture’ or ‘culture incubator’ or ‘cumulus coculture’ or ‘blastocyst culture technique’ or ‘blastocyst media’ or ‘media’ or ‘G1’ or ‘G1.2’ or ‘G2’ or ‘G2.2 sequential’ or ‘Medicult’ or ‘Medicult Sequential Medium’ or ‘Vitrolife’ or ‘sequential culture’ or ‘sequential media’ or ‘fetal bovine serum’ or ‘fetal cord serum’ or ‘P1’ or ‘P1 culture medium’ or ‘human tubal fluid’ or Title CONTAINS ‘embryo culture’ or ‘embryo culture media’ or ‘Culture-Media’ or ‘culture’ or ‘culture incubator’ or ‘cumulus coculture’ or ‘blastocyst culture technique’ or ‘blastocyst media’ or ‘media’ or ‘G1’ or ‘G1.2’ or ‘G2’ or ‘G2.2 sequential’ or ‘Medicult’ or ‘Medicult Sequential Medium’ or ‘Vitrolife’ or ‘sequential culture’ or ‘sequential media’ or ‘fetal bovine serum’ or ‘fetal cord serum’ or ‘P1’ or ‘P1 culture medium’ or ‘human tubal fluid’). Since the first papers comparing culture media for human IVF/ICSI were published in 1985, our search strategy starts from 1985. More detailed information on search strategies is available in Supplementary data, Table SI. A Cochrane review on this subject will be developed and it will be updated regularly (Youssef et al., 2009).

The citation lists of relevant review articles and included studies were also searched. Conference abstracts from the annual meetings of the European and American societies on human reproduction (ESHRE and ASRM) from 1985 to 2012 were manually searched. Authors of included studies were contacted for any additional information about their study when necessary. RCTs that compared commercially available media for the in vitro culture of human preimplantation embryos were included without any limitation to language.

All identified articles were independently assessed by two investigators (E.M. and M.A.F.M.Y.) and disagreements were discussed further with a third investigator (S.R.). Quality assessment on the included studies was based on the recommendations of the Cochrane Handbook of Systematic reviews and was performed by one of the authors and checked by the other (Higgins and Green, 2011). The overall study quality was assessed as good (+) if the study fulfilled the key requirements of a randomized control trial (allocation concealment, blinding and randomization), moderate (±) if the study fulfilled some of requirements and poor (−) if the study fulfilled none of the above requirements or if the study design regarding these parameters was unclear. In case no data were available for any of the studied outcomes, the primary author of the study was contacted and if there was no response the paper was excluded.

More recently, sequential culture media were designed to take into account the changing metabolic needs of the embryo from the cleavage to the blastocyst stage (Gardner and Lane, 1997).

Despite all these changes in culture media, it is still unclear whether the composition of the media affects embryo quality and IVF/ICSI success rates and which culture medium leads to the best IVF/ICSI success rates.

Here, we present a systematic review of randomized controlled trials (RCTs) describing the effect of embryo culture media on IVF/ICSI success rates.

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**Culture media for human preimplantation embryos**

(Bungum et al., 2002; Cooke et al., 2002; Friedler et al., 2007). Currently, many culture media are commercially available, each with a different composition. The first culture media in IVF/ICSI were balanced salt solutions to which glucose and phosphate were added. Subsequently, more complex culture media formulations with the addition of non-essential amino acids, chelators (EDTA), vitamins and antibiotics were introduced (Gardner et al., 1994; Gardner and Lane, 1996). More recently, sequential culture media were designed to take into account the changing metabolic needs of the embryo from the cleavage to the blastocyst stage (Gardner and Lane, 1997).

Characteristics of included studies

Five hundred and sixty-six potentially relevant abstracts were identified by our search strategy. After reading the abstracts, 461 abstracts were excluded because they were comparing early versus late embryo transfer, because they were comparing media supplementation or because they were studying different species. Of the remaining 105 abstracts that appeared to meet the inclusion criteria, the full papers were retrieved. Another 65 papers were excluded after in-depth assessment of presented data. Reasons for exclusion were: the trial was not relevant to the topic under study (25), the trial reported incomplete data and no response was received after contacting the corresponding author (18), the trial was not an RCT (14), the trial was a republication of a previous trial (7) and the trial appeared to be a review (1) (Fig. 1 and Supplementary data, Table SII). Further, 18 studies were excluded because they were published as abstracts in...
conference books more than 5 years ago and were never published in peer reviewed journals (Supplementary data, Table III). Thus, 22 trials were included in our review (Quinn et al., 1985; Parinaud et al., 1998, 1999; Staessen et al., 1998; Mauri et al., 2001; Utsunomiya et al., 2002; Artini et al., 2004; Ben-Yosef et al., 2004; Findikli et al., 2004; Summers-Chase et al., 2004; Zollner et al., 2004; Balaban and Urman, 2005; Hoogendijk et al., 2007; Reed et al., 2009; Sepulveda et al., 2009; Campo et al., 2010; Dumoulin et al., 2010; Hambiliki et al., 2010; Paternot et al., 2010; Di Falco Cossiello et al., 2011; Khoury et al., 2012; Nelissen et al., 2012).

The basic characteristics (number of women, oocytes and embryos, mean female age, mean number of embryos transferred, whether it was a multicenter study, whether donor oocytes were included, time of embryo transfer and whether frozen embryos were included) are shown in Table I. Fifteen studies randomized women or cycles, six studies randomized oocytes and one study randomized embryos. All studies were performed at single private or university-based clinics apart from three studies for which the location in which they were performed was unclear. IVF was performed in three studies, ICSI was performed in five and the other studies used both IVF and ICSI. Three studies reported on donor and non-donor oocytes, one only on donor oocytes and the rest of the studies used non-donor oocytes. Twelve studies performed early embryo transfer (Day 2–3), four studies performed late embryo transfer (Day 4–6) and six studies performed transfers on Day 3 or Day 5. Three studies transferred fresh as well as frozen-thawed embryos and all other studies transferred fresh embryos only.

The quality of the included studies (whether it was a full paper or not, allocation concealment, blinding, method of randomization, power calculation and whether it was an intention-to-treat analysis) is shown in Table II. There were 21 studies published as full papers and 1 published as abstract. Seven studies reported concealment of allocation, four studies reported no concealment while in the rest of the studies it was unknown whether concealment was performed. Blinding was performed in six studies, five studies reported no blinding and for the rest of the studies it was unclear. Two studies randomized women using a computer program (Ben-Yosef et al., 2004; Di Falco Cossiello et al., 2011), two used sealed envelopes (Campo et al., 2010; Paternot et al., 2010), one randomized by drawing lots (Mauri et al., 2001) and the compared culture media were used alternately in nine studies, while for the remaining studies the exact method of randomization was not reported. One study randomized patients according to patient number for a part of the study and according to oocytes in a second part of the study (Khoury et al., 2012).

The reported comparisons and outcomes per study are shown in Table III. In our analysis, pregnancy outcomes from trials that randomized oocytes or embryos were excluded because of the difference in the unit of analysis. From trials that randomized women (or cycles), both outcomes that are analyzed per woman (or cycle) and outcomes that are analyzed per oocyte or embryo were included. Many studies did not report all data necessary for analysis; in this case, the principal investigators of the studies were contacted for additional information. Of the 22 contacted authors, 11 responded to our questions. When we received no answer from the authors, we included the studies only for the outcomes for which data were available.

Characteristics of the culture media used

There were 20 different culture media from 11 commercial companies represented in the review. These companies were: Irvine Scientific (HTF, P1, MultiBlast); Vitrolife (G2, G3, G5 series); MediCult (Universal IVF, BlastAssist, ISM, EmbryoAssist); Cook (Sydney IVF cleavage/blastocyst media); Biopharma Sage (Quinn’s Advantage); Scandinivian IVF (IVF); InVitroCare (HTF); IVF Online (Global), Elilos Bio-Media (EllioStep2, BM1, SMART2), Api-System (Menezo B2) and Gynemedia (GM501). G series media were used in eight studies, Sydney IVF in six, HTF, Global and P1 in four studies each, Quinn’s Advantage and IVF media in three studies each, MultiBlast, BM1, ISM, Universal IVF, GM501 and EllioStep2 media in two studies each and the other media were used in one study each. The comparisons among the various culture media are shown in Fig. 2. All studies involved individual comparisons between two media apart from P1 versus HTF and Sydney IVF versus G3 that were compared in two studies each.

Pregnancy outcomes

An overview of all available pregnancy data is provided in Fig. 3. For each outcome, results are sorted based on the effect size.

Four studies reported on live birth rate: two after Day 2–3 embryo transfer and two in both subgroups. No evidence of a statistical difference was observed between the compared media apart from one study where significantly more live births were observed in embryos cultured in G3 compared to embryos cultured in Sydney IVF [risk difference (RD) 0.26, 95% confidence interval (CI): (0.02, 0.11)] (Nelissen et al.,
One of the included studies reported on the birthweight of babies born (Nelissen et al., 2012). This study showed that embryos cultured in Sydney IVF resulted in singletons with lower birthweight compared with embryos cultured in G3.

Four studies reported on ongoing pregnancy rate: one after Day 2–3 embryo transfer, two after Day 4–6 embryo transfer and one in both subgroups. The gestation weeks up to when an ongoing pregnancy is defined were unclear in most of the studies. One study comparing three media with each other found a significant difference in ongoing pregnancies after late embryo transfer (Utsunomiya et al., 2002).

Culture in both MultiBlast and Sydney IVF resulted in more ongoing pregnancies compared with culture in G2 medium \([\text{RD} 0.26, \text{CI} (0.08, 0.44)]\) and \([\text{RD} 0.26, \text{CI} (0.10, 0.42)]\), respectively.

Nine studies reported on clinical pregnancy rate: four of them after Day 2–3 embryo transfer, two studies after Day 4–6 embryo transfer and three in both subgroups. Six comparisons were found to be significantly different. For early embryo transfer, HTF resulted in more clinical pregnancies compared to G2 \([\text{RD} 0.19, \text{CI} (0.01, 0.21)]\) and G3 resulted in more clinical pregnancies than Sydney IVF \([\text{RD} 0.06, \text{CI} (0.01, 0.10)]\). For late embryo transfer, MultiBlast and Sydney IVF resulted in more clinical pregnancies compared to G2 \([\text{RD} 0.26, \text{CI} (0.08, 0.44)]\) and \([\text{RD} 0.26, \text{CI} (0.10, 0.42)]\), respectively.

G3 resulted in more clinical pregnancies than G2 both after early and late embryo transfer \([\text{RD} 0.12, \text{CI} (0.03, 0.22)]\) and \([\text{RD} 0.14, \text{CI} (0.00, 0.27)]\), respectively.

Seven studies reported on miscarriage rate: three after Day 2–3 embryo transfer, two after Day 4–6 embryo transfer and two after both time points. Three comparisons were found to be statistically significant. BlastAssist, MultiBlast and Sydney IVF all three resulted in more miscarriages compared with G2 \([\text{RD} 0.10, \text{CI} (0.01, 0.19)]\), \([\text{RD} 0.14, \text{CI} (0.02, 0.26)]\) and \([\text{RD} 0.10, \text{CI} (0.00, 0.20)]\), respectively.

Five studies reported on multiple-pregnancy rate: one for Day 2–3 embryo transfer, one for Day 4–6 embryo transfer and three both for Day 2–3 and Day 4–6 embryo transfer. G3 medium resulted in a higher multiple-pregnancy rate compared with G2 after early embryo transfer, but the difference was not significant after late embryo transfer \([\text{RD} 0.11, \text{CI} (0.03, 0.18)]\) and \([\text{RD} 0.09, \text{CI} (0.00, 0.20)]\), respectively.

We performed an unconventional hypothesis-generating meta-analysis on pregnancy rates per woman for five media that were involved in multiple comparisons. Each of these media was compared with the pooled data of the combined comparisons (Fig. 4). Only one

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**Table 1 Baseline characteristics of included studies.**

<table>
<thead>
<tr>
<th>Included studies</th>
<th>Women/oocytes</th>
<th>Age (mean)</th>
<th>ET (mean)</th>
<th>Multicenter</th>
<th>IVF/ICSI</th>
<th>Donor oocytes</th>
<th>Time of embryo transfer</th>
<th>Fresh/frozen embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinn et al. (1985)</td>
<td>113</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>IVF</td>
<td>No</td>
<td>Early</td>
<td>Fresh</td>
</tr>
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<td>Parinaud et al. (1998)</td>
<td>416</td>
<td>–</td>
<td>2.8</td>
<td>–</td>
<td>ICSI</td>
<td>No</td>
<td>Early</td>
<td>Fresh</td>
</tr>
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<td>ICSI</td>
<td>No</td>
<td>Early/late</td>
<td>Fresh</td>
</tr>
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<td>IVF/ICSI</td>
<td>No</td>
<td>Late</td>
<td>Fresh</td>
</tr>
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<td>33</td>
<td>3</td>
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<td>IVF/ICSI</td>
<td>No</td>
<td>Early/late</td>
<td>Fresh</td>
</tr>
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<td>Fresh</td>
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<td>Early</td>
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<td>Early/late</td>
<td>Fresh/frozen</td>
</tr>
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<td>39</td>
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<td>–</td>
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<td>ICSI</td>
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<td>Early/late</td>
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</tr>
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<td>–</td>
<td>No</td>
<td>IVF/ICSI</td>
<td>No</td>
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<table>
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<tr>
<th>Studies randomizing oocytes/embryos</th>
<th>Women/oocytes</th>
<th>Age (mean)</th>
<th>ET (mean)</th>
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<th>IVF/ICSI</th>
<th>Donor oocytes</th>
<th>Time of embryo transfer</th>
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<td>IVF</td>
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<td>IVF/ICSI</td>
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<td>Fresh</td>
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<td>Findiki et al. (2004)</td>
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<td>–</td>
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<td>Late</td>
<td>Fresh</td>
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<td>37.4</td>
<td>2.3</td>
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<td>IVF/ICSI</td>
<td>yes</td>
<td>Early/late</td>
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<td>Hambili et al. (2010)</td>
<td>1206</td>
<td>33.9</td>
<td>–</td>
<td>No</td>
<td>IVF</td>
<td>No</td>
<td>Early</td>
<td>Fresh</td>
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<td>Di Falco Cossiello et al. (2011)</td>
<td>2289</td>
<td>33.6</td>
<td>2.2</td>
<td>No</td>
<td>ICSI</td>
<td>Yes</td>
<td>Early</td>
<td>Fresh</td>
</tr>
</tbody>
</table>

ET, embryos transferred; –, unknown.
culture medium (G2) resulted in significantly less pregnancies compared with the group of other media [RD \(-0.12\), 95% CI: \((-0.22, -0.03)\)], but this result should be interpreted with caution as the heterogeneity among the studies was high \(I^2 = 57\%). For the other four culture media, no significant differences were found.

**Embryo outcomes**

An overview of all available embryo data is provided in Fig. 5. For each outcome, results are sorted based on the effect size.

Ten studies reported on fertilization rate (the definition of fertilization was heterogeneous among the studies) while studies that transferred the embryos to the compared media after assessment of fertilization were excluded for this outcome measure. Two comparisons showed a significant difference. GM501 resulted in a higher fertilization rate compared with Sydney IVF [RD 0.11, 95% CI: (0.07, 0.16)] and G2 resulted in a higher fertilization rate compared with BlastAssist [RD 0.08, 95% CI: (0.04, 0.11)].

Given that the assessment and reporting (mean, average or percentage) of embryo quality was very heterogeneous, only eight studies that reported on the number of good quality embryos could be included in our analysis. Eight comparisons showed a significant difference. Global resulted in more good quality embryos compared with Universal IVF, HTF and IVF media [RD 0.29, 95% CI: (0.21, 0.37), RD 0.19, 95% CI: (0.12, 0.26) and RD 0.15, 95% CI: (0.08, 0.23), respectively]. Quinn’s advantage and P1 resulted in a higher rate than HTF [RD 0.12, 95% CI: (0.08, 0.16) and RD 0.07, 95% CI: (0.02, 0.11), respectively], P1 resulted in a higher rate than Sydney IVF [RD 0.04, 95% CI: (0.01, 0.07)] and G3 resulted in a higher rate compared with G2 [RD 0.12, 95% CI: (0.09, 0.15)] and Sydney IVF better than GM501 [RD 0.10, 95% CI: (0.04, 0.16)].

Three studies reported on cryopreservation rate: two after Day 2–3 embryo transfer and one after Day 4–6 embryo transfer. None of the comparisons resulted in a statistically significant difference.

Fifteen studies reported on implantation rate: 10 after 2–3 days of culture, three studies reported on implantation rate after Day 4–6 embryo transfer and two studies reported on implantation rate after both Day 2–3 and 4–6 embryo transfer. Six comparisons resulted in statistically significant differences after early embryo transfer and four comparisons gave significant differences in the implantation rate after late embryo transfer. After early embryo transfer, BM1 resulted in a higher implantation rate than MB2 [RD 0.34, 95% CI: (0.05, 0.63)], Quinn’s advantage and P1 resulted in a higher rate than HTF [RD 0.12, 95% CI: (0.08, 0.16) and RD 0.07, 95% CI: (0.02, 0.11), respectively], P1 resulted in a higher rate than Sydney IVF [RD 0.04, 95% CI: (0.01, 0.07)] and G3 resulted in a higher rate compared with G2 and Sydney IVF [RD 0.11, 95% CI: (0.07, 0.16) and RD 0.10, 95% CI: (0.03, 0.17), respectively]. After late embryo transfer, Global resulted in a higher implantation rate compared with MultiBlast [RD 0.21, 95% CI: (0.05, 0.36)] and G3, Sydney IVF and MultiBlast resulted in higher rate compared with G2 [RD 0.16, 95% CI: (0.02, 0.30), RD 0.09, 95% CI: (0.02, 0.14), respectively].

**Discussion**

In this systematic review, we assessed the effect of commercially available culture media on IVF/ICSI success rates to indentify the medium...
with the best clinical outcomes. We were unable to identify such a medium, due to the paucity of data available in the literature. The 22 included studies involved different comparisons or reported data in such a way that proper meta-analysis was not possible for neither primary nor secondary outcome measures. Only four studies reported on live births and one found a significant difference. Although meta-analysis was not possible, in the majority of the studies, a difference in pregnancy rate of more than 5% (RD ≥ 0.05) between the culture media was observed, indicating the clinical relevance of culture media for IVF/ICSI success rates. We were able to perform an unconventional hypothesis-generating meta-analysis by combining data from studies that shared the same culture medium in one of the treatment arms. Out of the five media that were part of this meta-analysis, G2 resulted in less pregnancies compared with the group of other media but this result is based on studies with high heterogeneity (I² = 57%) so it should be cautiously interpreted.

The overall quality of the included studies was low. Only four studies reported on live birth rate per woman and four on ongoing pregnancy rate while it is commonly accepted that at least ongoing pregnancy and preferably live birth should be the primary outcome of clinical studies to assess IVF/ICSI success rates (Barlow, 2003;...
The majority of the included studies had methodological limitations such as a weak randomization protocol, randomization of oocytes and embryos rather than women, small sample size and absence of a power calculation. In addition, not all studies commented on all outcomes and many studies reported part of their outcomes as percentages or as a proportion.

Figure 3 Overview without meta-analysis of the pregnancy outcomes of all included studies where women or cycles (*) were randomized. Women or cycles are used as unit of analysis in this overview. For each outcome studies are sorted based on effect size.
means without providing exact numbers thereby preventing extraction of relevant data. This was also the case for 22 trials that had to be excluded since no relevant data could be extracted at all. Exact definitions of pregnancy (clinical and ongoing), miscarriages, and fertilization were not provided which could result in discrepancies among the studies. Finally, the way the embryo quality was reported was very heterogeneous with some studies providing the number of good quality embryos as percentages, means or averages without providing exact numbers. In addition, the way embryo quality was scored varied greatly among the different studies. This heterogeneity led to exclusion of much potentially interesting data.

In our analysis, we excluded pregnancy data from studies that randomized oocytes and embryos. This decision was based on several reasons. First, by using this data from trials randomizing embryos and oocytes, a unit of analysis error is introduced. Secondly, in case of oocyte and embryo randomization, oocytes and embryos from one patient are randomized between the compared media but only the best morphological embryos will be selected for transfer. This design introduces bias since the morphology of the embryos that are transferred might depend on the medium used. Lastly, the analysis of the clinical outcomes is based on the number of women and it is impossible to calculate the live birth and pregnancy rates per woman from trials that analyzed outcomes per oocyte or embryos. Embryo data from trials that randomized patients were included in the analysis. The potential confounding effect of including multiple oocytes/embryos from one woman/cycle was neglected as these concerned only secondary outcomes of our review and since only very limited data would otherwise be available for review.

To clarify whether culture media do have an effect on IVF/ICSI success rates and to determine the magnitude of such an effect, more good quality trials need to be conducted. The data presented in this review can be used as a guideline for researchers planning to conduct such RCTs on different culture media in the future. Changing the scope from embryological to clinical outcomes, performing proper randomization methods to ensure blinding and allocation concealment, and clear data reporting by providing exact numbers together with percentages or means will lead to better quality studies being available to be used for meta-analysis.

It should be noted that some of the media described in the included studies, such as ElioStep, BM1, SMART2, Menezo B2, T6 or the G2 and G3 series of Vitrolife, are no longer used for human IVF. Of importance is the fact that even today new culture media are introduced into clinical care without properly designed RCTs. Obviously there are commercial aspects to this as large scale RCTs are both time and money consuming. Moreover, if an RCT indicates equal or even lower success rates than already available media, years of research and development are wasted. Nevertheless, it is our firm belief that such an essential component of IVF should be treated with the highest level of scrutiny and that companies should report what studies have been performed and which endpoints were analyzed upon introduction of new media.

Only one of the studies reported data on neonatal outcomes. Recent studies have indicated that the type of culture media used to culture human preimplantation embryos can affect birthweight of newborns (Dumoulin et al., 2010). In addition, animal data suggest that the type of culture media used can affect gene expression and the imprinting status of bovine and mouse embryos (Lonergan et al., 2006; Fernandez-Gonzalez et al., 2009; Market-Velker et al., 2010). This suggests that the in vitro culture of human embryos might have prolonged effects on the health of offspring, similar to the effect of in utero under-nutrition on disease susceptibility in adulthood or the effect of in vitro maturation media on human oocytes and embryos (de Rooij et al., 2007; Schulz 2010; Ben-Ami et al., 2011). We therefore suggest that next to the live birth rate, also the health of offspring should be included in future RCTs investigating culture media.

Retrospective studies have been excluded from our analysis as their design is inferior to that of prospectively randomized studies (Aoki et al., 2005; Xella et al., 2010; Wirleitner et al., 2010; Eaton et al., 2012; Vergouw et al., 2012). These retrospective studies, like the randomized studies that are included in our analysis, report on different comparisons and different outcomes. Of interest are two studies that investigated the effect of culture media on the neonatal birthweight (Eaton et al., 2012; Vergouw et al., 2012). In contrast to the study of Nelissen et al. (2012) that reported a significant difference in birthweight between Sydney IVF and G3, these studies found no significant differences in birthweight for embryos cultured in G3, Global and G5 media or HTF and Quinn’s advantage respectively.

It should also be mentioned that other factors during embryo culture could influence IVF/ICSI success rates. Such factors include the number of embryos per drop and culture dish and embryo produced factors (Hoelker et al., 2010). None of the included studies reported explicitly on these factors.

This systematic review shows an effect of culture media for human preimplantation embryos on embryo quality and success rates during IVF/ICSI treatment cycles. The existing data, especially on ongoing pregnancies and live births, are insufficient to allow the selection of the best culture medium for IVF/ICSI and thus more rigorously

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**Figure 4** Meta-analysis of clinical/ongoing pregnancy rates. Medium B refers to all other media with which medium A has been compared.
designed RCTs are necessary for both currently used culture media as well as newly introduced culture media.

**Supplementary data**


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**Figure 5** Overview without meta-analysis of the embryological outcomes of all included studies. Oocytes or embryos are used as unit of analysis in this overview. The asterisk indicates studies that have randomized women instead of oocytes or embryos. + indicates a study that randomized both oocytes and women. For each outcome studies are sorted based on effect size.

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**Authors’ roles**

E.M.: design of study; acquisition, analysis and interpretation of data; drafting and revising the manuscript; final approval of manuscript. M.A.F.M.Y.: conception and design of study, acquisition, analysis and interpretation of data, revising the manuscript; final approval of manuscript. M.W., F.V., H.G.Ali-I.: interpretation of data; revising the manuscript; final approval of the manuscript. S.R., S.M.: conception of study; interpretation of data; revising the manuscript; final approval of the manuscript.

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