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The Effect of Two Different Embryo Culture Media on Birthweight of the Offspring

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ABSTRACT

Purpose: To investigate the effect of medium type on neonatal birthweight in singleton, term infants conceived following assisted reproductive technology (ART).

Methods: The records of 352 patients who gave birth after in vitro fertilization (IVF) treatment from January to December 2014 in the Eurofertil IVF Center using a time-lapse embryo culture system were analyzed. Data analysis was performed using two available culture media: Vitrolife (n=267) and MediCult (n=85).

Results: The mean birthweight of the infants from Vitrolife cultures was 3006 grams, though it was 3128 grams for the offspring from MediCult (p=0.154). There was no significant difference between neonates born using either medium in terms of birthweight.

Conclusion: There was no association between neonatal birthweight after ART and the *in vitro* embryo culture media used in this population. These findings are important in order to assess human embryo *in vitro* culture safety. To evaluate if culture media affects prenatal outcome and development, more randomized, controlled studies would be needed.

Keywords: Birthweight, embryo, fertilization in vitro

INTRODUCTION

The most frequently used treatments for subfertility are in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Over the past forty years, significant advancements and enhancements have been made to assisted reproductive technology (ART).[1] From early simple balanced salt solutions to later complex culture systems containing multiple components like phosphate, vitamins, amino acids, lipids, trace elements, and other biomolecules, the medium underwent a gradual optimization process.[2] Without knowledge of the optimal microenvironment for embryo development *in vivo*, we are unable to ascertain the most suitable circumstances for cultivating embryos in the laboratory.

It has been extensively documented that newborns conceived through ART are more prone to preterm birth and low birthweight when compared to infants born naturally.[3] This has been attributed to both inherent individual features and particular characteristics of the IVF procedure.[4] In addition, other well-known factors that influence neonatal outcome are maternal body mass index (BMI), smoking and alcohol consumption.[5] Moreover, there may be a correlation between gender and elevated birthweights; males tend to have greater birthweights than girls. Furthermore, both parity and gestational age are associated with an increase in birthweight.[6]

During preimplantation development, there is a process termed "epigenetic reprogramming" that involves deleting gametic alterations and establishing embryonic epigenetic modifications. ART has the potential to modify these modifications, including the potential influence of the diverse composition of the culture media employed during *in vitro* embryo development.[7] Different reports indicate that the culture media utilized in IVF embryo culture have distinct impacts on perinatal outcomes. In addition, the success rate and treatment results of IVF/ICSI operations depend on culture media choice. Selection of embryo culture medium affects embryo quality and conception rates.[8]

Since the inception of IVF as an ART, various culture systems have been used for the cultivation of human embryos. For optimal development, the chemical composition of the media used *in vitro* to cultivate human embryos is critical. There is no growth medium that perfectly replicates the conditions *in*

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vivo, so embryos that are grown in a laboratory are always stressed in some way. The embryo culture medium used in IVF laboratories may have an impact on birthweight and other outcomes for infants.[9] Animal studies have shown that particular components of the culture media, and changes in the concentration of these components, may lead to changes in birthweight of offspring.[10]

An important randomized controlled trial (RCT) observed a mean disparity of 158 g in birthweight between two distinct media sources.[11] The substantial value of the evidence gathered from RCTs supports the hypothesis that IVF culture media affect the in utero development, weight, and adiposity of the neonate at the age of nine.[12]

The impact of medium type on birthweight in human IVF remains mostly unknown. Furthermore, a recent systematic review revealed that there are few randomized studies comparing the clinical outcomes of various culture media, and those that do have unsound methodology.[13,14] Thus, this topic remains plagued by unanswered questions, and the debate continues. Given the significant predictive value of birthweight for long-term health consequences, it is important to determine whether particular culture media are associated with adverse fetal outcomes that may have enduring effects in maturity.

In this study the effects of two different commercial embryo culture media (MediCult, Denmark vs. Vitrolife, Sweden) on birthweight were investigated.

METHODS

Subjects

In the present study, the records of patients who gave birth after IVF treatment at the Eurofertil IVF Center from January to December 2014, using a time lapse embryo culture system, were analyzed by the end of 2015. The research protocol was approved by the Ethics Review Committee of Bahçeşehir University.

Maternal age, maternal BMI, paternal age, cycle type (agonist, antagonist, Femara, thaw, natural cycle), estradiol (E2) levels and gonadotropin intake status and sperm preparation methods were evaluated for both media. Pregnancy and infant characteristics (gestational age, newborn gender, twin pregnancy rates and mean birthweight) were compared between the two media. Only the data from the initial IVF treatment cycles of all patients treated at our center during the research period were selected for the present study. Patients requiring preimplantation genetic diagnosis and pregnancies resulting in significantly abnormal birthweight (<2500 g and >4500 g) were excluded. Among the cases receiving IVF treatment during the study period only those cases having clinical pregnancy diagnosed with transvaginal ultrasound were included.

Laboratory Protocols

Embryos derived from a fresh IVF/ICSI cycle were cultured either in MediCult (Jyllinge, Denmark) or Vitrolife (Gothenburg, Sweden). Subsequently, the laboratory's routine insemination protocols were followed for the IVF and ICSI procedures. Fertilized oocytes were placed in microwells (Mannheim, Germany) with 35 mL culture medium and 1.4 mL oil. A time-lapse monitoring system with an incubator and optical microscope automatically captured images until transfer. Five focal planes were used to record embryo growth every 10 minutes.[15] An assessment of the morphology of the embryos was conducted 68-72 hours post-inoculation, focusing on cell count, fragmentation and symmetry. The criteria for scoring were fragmentation, grade, and cell number. Good quality 72-hour embryos were distinguished by the presence of 7-9 mononucleated blastomeres of equal size, with fragmentation below 10%.

Embryo Transfer Protocols

The number of embryos to transfer was determined based on each patient's age, medical history, and the characteristics of the available embryos. Embryo transfer (ET) was performed on days 2, 3, or 5 after oocyte retrieval, depending on embryo quantity and quality. The selection of embryos for transfer was based on their developmental quality, determined by assessing their morphokinetics and ultimate morphological appearance. The serum ß-human chorionic gonadotropin (β-hCG) level was assessed 12 days post ET. An ultrasound was conducted 14 days following a positive β-hCG test to determine the presence of gestational sac(s). The assessment of clinical pregnancy occurred between 7 and 14 days after the identification of the gestational sac, through the detection of fetal cardiac heartbeat.

Statistical Analysis

The data were subjected to statistical analysis utilizing SPSS, version 23.1 (IBM Inc., Armonk, NY, USA). After fresh ETs, the mean values of patient baseline characteristics were analyzed across the two categories of culture media using chi-square testing for categorical and continuous variables and Student's t-tests for continuous variables. A significance level of two-sided p-values below 0.05 was regarded as statistically significant.

RESULTS

During the study period there were 815 couples receiving IVF treatment. The clinical pregnancy rate was 50.7% (414/815). There were 47 (11.3%) cases of abortion before 20 weeks of gestation, nine cases lost to follow-up due to telephone or address change and six cases of intrauterine deaths, leaving 352 patients with live birth for analysis. There were 312 singleton births and 41 sets of twins.

Based on the culture medium employed, Table 1 shows the key maternal, paternal, and treatment characteristics of the two groups. Age, BMI, paternal age, cycle type, sperm preparation method, and gonadotropin utilized in the treatment were all criteria that did not show a significant difference between the two groups that were given cultured medium. In Table 2, cycle type (agonist, antagonist, Femara, dissociative, natural cycle), E2 levels and gonadotropin uptake status and sperm preparation methods were evaluated for both media. It has been shown that cycle type, gonadotropin intake and sperm preparation method are significant in terms of outcome ($p<0.05$).

Table 1. Demographic data of the parents

Variable	Group 1 (Vitrolife) (n=612)	Group 2 (MediCult) (n=203)	p-value
Maternal age (years)	31.2±4.9	30.8±5.1	0.3
Maternal BMI (kg/m^2)	23.8±4.1	24.4±3.9	0.06
Paternal age (years)	33.1±5.2	32.7±5.3	0.34
Basal FSH	7.0±2.6	7.1±2.3	0.62
Previous ICSI cycles	1.7±1.3	1.9±1.4	0.06
Cause of infertility			
Unexplained	202 (33)	71 (35)	0.2
Male factor	269 (43.9)	73 (36)	-
PCOS	37 (6)	14 (6.8)	-
Endometriosis	55 (8.9)	18 (8.8)	-
Tubal factor	36 (5.8)	20 (9.8)	-
Azoospermia	13 (2.1)	7 (3.4)	-

The data are presented as n (%) or mean ± SD from the mean.
BMI: Body mass index, FSH: Follicle stimulating hormone, ICSI: Intracytoplasmic sperm injection, PCOS: Polycystic ovary syndrome, SD: Standard deviation

Table 2. Cycle characteristics and pregnancy outcome

Variable	Group 1 (Vitrolife) (n=612)	Group 2 (MediCult) (n=203)	p-value
Cycle type			
Agonist	9 (1.4)	1 (0.4)	<0.01*
Antagonist	459 (75)	119 (58.6)	-
Letrazole only	7 (1.1)	1 (0.4)	-
Thaw cycle	134 (21.2)	81 (39.9)	-
Natural cycle	3 (0.4)	1 (0.4)	-
E2 (pg/mL)	2175±941	2075±1325	0.2
Gonadotropin (450 IU/0.75 mL)			
Recombinant FSH	325 (53.1)	132 (65)	<0.01*
Urinary FSH	18 (2.9)	8 (3.9)	-
FSH+LH	269 (43.9)	63 (31)	-
Sperm preparation method			
Swim up	466 (76)	131 (64.5)	<0.01*
Gradient	74 (12)	16 (7.8)	-
Sperm wash	72 (11.7)	56 (27.5)	-
MII oocytes aspirated	10.8±5.4	10.2±4.7	-
Clinical pregnancy rate	318 (51.9)	96 (47.2)	0.2
Spontaneous abortion rate	36 (5.9)	11(5.4)	0.8
Lost to follow-up	7 (1.1)	2 (0.9)	0.8
Take home baby rate	267 (43.6)	85 (41.8)	0.6

***p<0.05**
The data are presented as n (%) or mean ± SD from the mean.
FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, MII: Metaphase II, SD: Standard deviation

Table 3. Pregnancy outcome and infant characteristics according to culture media used

	Group 1 (Vitrolife) (n=267)	Group 2 (MediCult) (n=85)	p-value
Gestational age (weeks)	37.19±0.19	37.89±0.17	0.045*
Gestational age category			
<32 (very preterm)	21 (8.0)	2 (2.4)	0.14
32 to 37 (preterm)	38 (14.5)	10 (11.9)	-
≥37	202 (77.5)	72 (85.7)	-
Newborn gender			
Male	141 (47.9)	40 (43.4)	0.71
Female	153 (52.1)	52 (56.6)	-
Twin	33 (12.3)	9 (10.5)	0.50
Not twin	234 (87.6)	76 (89.4)	-
Mean birthweight of singletons (g)	3006±44	3137±58	0.13
Mean birthweight of twins (g)	2285±106	2323±126	0.86
Birthweight category			
<1500 g (very low)	15 (5.6)	1 (0.1)	0.35
<2500 g (low)	34 (12.7)	10 (11.7)	-
2500-4000 g (normal)	210 (78.6)	72 (84.7)	-
>4000 g (high)	8 (2.9)	2 (2.3)	-

*p<0.05

The relationship between medium used and birthweigth is shown in Table 3. Mean birthweight of the singleton offspring from pregnancies using Vitrolife was 3006 g while it was 3137 g in pregnancies using MediCult (p=0.154). Similarly, the mean twin birthweight was 2285 g for twins in the Vitrolife group and 2323 g for twins in the MediCult group (p=0.86). There was no significant difference in mean birthweight between the births using either medium type.

Table 3 shows parameters of delivery and offspring depending on the culture medium used. Parameters compared include gestational age, gender of the offspring, and mean birthweight. The only significant difference was found for gestational age which was significantly older in the MediCult group.

DISCUSSION

This retrospective study demonstrated that the birthweight of the offspring was not significantly affected by using either Vitrolife or MediCult, two distinct embryo culture media for IVF.

Birthweight is widely recognized as a prevalent indicator used for perinatal outcome assessment, which has been integrally linked to morbidity and mortality.[16] Embryonic development during IVF is dependent upon culture medium. Thus, many IVF centers have considerd the impact of *in vitro* embryo cultivation on neonatal birthweight. Nevertheless, there is variation among manufacturers regarding the composition and concentrations of nutrients present in various culture media.[13]

In terms of human IVF, the effect of medium choice on birthweight remains incompletely understood. Studies by Nelissen et al.[8] and Dumoulin et al.[9] showed that the kind of embryo culture medium used has an important effect on early embryonic development, fetal growth and birthweight of the baby. Dumoulin et al.[9] showed that singletons produced fresh

cleavage Day 2/3 ETs and grown in Vitrolife medium were heavier than those created through Cook medium culture. Using a small-scale cohort study involving both fresh and frozen ETs, he further supported his findings.[8] The disparity in growth became apparent as early as the second trimester of pregnancy and persisted for a minimum of two years after birth. Since then, research comparing various culture media has both confirmed and refuted these earlier conclusions.[8] Furthermore, birthweight after embryo culture in three different media and birthweight following spontaneous conception were compared in a relatively recently published Norwegian study, and the results revealed the birthweight as well as the placental weight differed between different culture media.[17] On the other hand, Eaton et al.[18] recently showed that there was no significant relationship between the embryo culture medium used and birthweight. Similarly, other studies using the same and other commercially available culture media did not find any changes in birthweight that were linked to using different embryo culture media.[18-20]

The results of the present study also suggest no significant association between culture medium and birth weight, in keeping with many earlier studies.[18-22] There remain the studies which have reported significant differences in birthweight in IVF neonates which have been attributed to the use of different culture media.[8,9,17]

Consistent with the results reported by Eaton et al.,[18] the current study's findings also indicate that birth weight and the culture medium employed did not correlate significantly in the 198 singleton deliveries analyzed. The results obtained in the investigation published by Vergouw et al.[19] were similar. Comparing two distinct culture media, the analysis of 358 singletons born after a fresh single ET and 159 singletons born after a frozen-thawed single ET revealed no significant difference in birth weight.

It has been reported that the use of Vitrolife medium resulted in a greater birth weight when compared to Cook culture media.[9] Upon analyzing 110 live singleton births from Vitrolife and 78 singleton births from Cook, the researchers discovered that the birth weight of the first group was significantly greater, both when adjusted for gender and gestational age. Notably, maternal and paternal weight, as well as height and weight, were significantly greater in the Vitrolife group than in the Cook group in this study. Nevertheless, Dumoulin et al.[9] conducted a retrospective analysis that encompassed singleton births occurring following fresh IVF-ICSI cycles, including those at >20 weeks gestation. This allowed for a comparison of neonates across a broader range of gestational ages.[9]

We felt it reasonable to compare MediCult and Vitrolife media because they differ in several components. MediCult incorporates synthetic serum replacement in the fertilization, cleavage, and blastocyst media. Vitrolife medium contains fructose, lactate, non-essential amino acids, and EDTA, whereas MediCult does not contain these components. Furthermore, the formulation of Vitrolife include methionine, hyaluronan, lipoic acid, and EDTA. The amino acid makeup of both blastocyst media is largely comparable, with the exception of arginine. Arginine is found in Vitrolife blastocyst medium, but not in MediCult blastocyst medium. Furthermore, both blastocyst media share four vitamins, but MediCult blastocyst medium additionally includes D-biotin, folic acid, and niacinamide. MediCult blastocyst medium includes inositol and ethanolamine, whereas Vitrolife blastocyst medium is fortified with hyaluronan.[23] The birthweight of infants raised as embryos in either of the two medium types did not vary, despite the significant component differences between two media. In this regard, the protein source in the culture media is also a significant factor since studies have indicated that its quality varies significantly across groups and manufacturers, and it has also been proposed that the protein source affects live birth rate and birthweight.[24] We hypothesize that the almost equal amounts of protein present in both culture media may be the reason for the lack of birthweight differences.

When analysing the culture media-mean birthweight relationship, many other factors should be taken into account. In the Cook and Vitrolife study groups, variations in parental characteristics, including maternal age, maternal and paternal BMI, maternal parity, and maternal smoking, may have affected the outcomes of the research conducted by Dumoulin et al.[9] and Nelissen et al.[8] In the present study maternal and paternal age and maternal BMI did not significantly differ between groups.[12]

Our retrospective study is limited in its design, a RCT would have been more informative. Another limitation of the present study is the heterogenous study populations and the differences in samples numbers for each medium. Thirdly, the influence of culture media on blastocyst transfer was not been investigated. The contentious impact of extended *in vitro* culture on birthweight was not considered.[25] Although this study only evaluated two types of culture media that are available, the findings can be integrated with other research that examine different commercially available culture media. This will expand the understanding of how various media types impact newborn outcomes.

Comparing the published studies on the impact of culture media on birthweight is challenging due to variations in study designs and research objectives. Moreover, human studies provide unique challenges in analysis when compared to animal studies. One of the major limitations is the frequent and consecutive use of various culture media. The majority of studies have employed a retrospective approach, using various culture media in consecutive time periods. This introduces a high likelihood of bias, as it is difficult to account for all variations in treatment protocols and population factors. Birthweight studies sometimes lacked crucial data on confounding factors, such as maternal smoking, parity, socio-economic status, sex distribution, and gestational age. We believe that other variables in our study population have the potential to influence the outcomes. Performing a multivariate regression analysis would have enhanced the validity of our hypothesis.

CONCLUSION

In summary, we found no correlation between the weight of neonates following ART and the choice of culture media,

MediCult versus Vitrolife, used for *in vitro* embryo culture. These results add to the evidence regarding the safety of *in vitro* culture of human embryos. They also offer reassuring data in comparison to previous studies that demonstrated an association between particular culture media and increased birth weight. Our findings are in keepig with previous studies that have demonstrated consistent birth weights, regardless of the embryo culture media used. While these findings offer some comfort, it is necessary to conduct bigger, randomized, controlled studies to determine whether various culture media have any impact on the prenatal outcome of the babies and their subsequent development.

Ethics

Ethics Committee Approval: The research protocol was approved by the Ethics Review Committee of Bahçeşehir University.

Informed Consent: Retrospectively study.

Authorship Contributions

Surgical and Medical Practices: E.E., Concept: H.Ö., Design: H.Ö., Data Collection or Processing: E.E., Analysis or Interpretation: E.E., Ö.A., H.Ö., Literature Search: Ö.A., Writing: E.E., Ö.A.,

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REFERENCES

1. Skakkebaek NE, Jørgensen N, Andersson AM, et al. Populations, decreasing fertility, and reproductive health. Lancet. 2019;393(10180):1500-1501.
2. Sunde A, Brison D, Dumoulin J, et al. Time to take human embryo culture seriously. Hum Reprod. 2016;31(10):2174-2182.
3. Henningsen AK, Pinborg A. Birth and perinatal outcomes and complications for babies conceived following ART. Semin Fetal Neonatal Med. 2014;19(4):234-238.
4. Pinborg A, Wennerholm UB, Romundstad LB, et al. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. Hum Reprod Update. 2013;19(2):87-104.
5. Odendaal HJ, Steyn DW, Elliott A, Burd L. Combined effects of cigarette smoking and alcohol consumption on perinatal outcome. Gynecol Obstet Invest. 2009;67(1):1-8.
6. Oken E, Kleinman KP, Rich-Edwards J, Gillman MW. A nearly continuous measure of birth weight for gestational age using a United States national reference. BMC Pediatr. 2003;3:6.
7. Velker BA, Denomme MM, Mann MR. Embryo culture and epigenetics. Methods Mol Biol. 2012;912:399-421.
8. Nelissen EC, Van Montfoort AP, Coonen E, et al. Further evidence that culture media affect perinatal outcome: findings after transfer of fresh and cryopreserved embryos. Hum Reprod. 2012;27(7):1966-1976.
9. Dumoulin JC, Land JA, Van Montfoort AP, et al. Effect of in vitro culture of human embryos on birthweight of newborns. Hum Reprod. 2010;25(3):605-612.
10. Young LE. Imprinting of genes and the Barker hypothesis. Twin Res. 2001;4(5):307-317.
11. Kleijkers SHM, Mantikou E, Slappendel E, et al. Influence of embryo culture medium (G5 and HTF) on pregnancy and perinatal outcome after IVF: a multicenter RCT. Hum Reprod. 2016;31(10):2219-2230.
12. Zandstra H, Brentjens LBPM, Spauwen B, et al. Association of culture medium with growth, weight and cardiovascular development of IVF children at the age of 9 years. Hum Reprod. 2018;33(9):1645-1656.
13. Mantikou E, Youssef MA, van Wely M, et al. Embryo culture media and IVF/ICSI success rates: a systematic review. Hum Reprod Update. 2013;19(3):210-220.
14. Youssef MM, Mantikou E, van Wely M, et al. Culture media for human pre-implantation embryos in assisted reproductive technology cycles. Cochrane Database Syst Rev. 2015;2015(11):CD007876.
15. Ergin EG, Calişkan E, Yalçinkaya E, et al. Frequency of embryo multinucleation detected by time-lapse system and its impact on pregnancy outcome. Fertil Steril. 2014;102(4):1029-1033.
16. Land JA. How should we report on perinatal outcome? Hum Reprod. 2006;21(10):2638-2639.
17. Eskild A, Monkerud L, Tanbo T. Birthweight and placental weight; do changes in culture media used for IVF matter? Comparisons with spontaneous pregnancies in the corresponding time periods. Hum Reprod. 2013;28(12):3207-3214.
18. Eaton JL, Lieberman ES, Stearns C, Chinchilla M, Racowsky C. Embryo culture media and neonatal birthweight following IVF. Hum Reprod. 2012;27(2):375-379.
19. Vergouw CG, Kostelijk EH, Doejaaren E, et al. The influence of the type of embryo culture medium on neonatal birthweight after single embryo transfer in IVF. Hum Reprod. 2012;27(9):2619-2626.
20. Carrasco B, Boada M, Rodríguez I, Coroleu B, Barri PN, Veiga A. Does culture medium influence offspring birth weight? Fertil Steril. 2013;100(5):1283-1288.
21. Lin S, Li M, Lian Y, Chen L, Liu P. No effect of embryo culture media on birthweight and length of newborns. Hum Reprod. 2013;28(7):1762-1767.
22. Ziebe S, Loft A, Povlsen BB, et al. A randomized clinical trial to evaluate the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) in embryo culture medium for in vitro fertilization. Fertil Steril. 2013;99(6):1600-1609.
23. De Vos A, Janssens R, Vande Velde H, et al. The type of culture medium and the duration of in vitro culture do not inşuence birthweight of ART singletons. Human Reproduction. 2015;30(1):20-27.
24. Zhu J, Li M, Chen L, Liu P, Qiao J. The protein source in embryo culture media influences birthweight: a comparative study between G1 v5 and G1-PLUS v5. Hum Reprod. 2014;29(7):1387-1392.
25. Fernando D, Halliday JL, Breheny S, Healy DL. Outcomes of singleton births after blastocyst versus nonblastocyst transfer in assisted reproductive technology. Fertil Steril. 2012;97(3):579-584.