



Influence of EmbryoGlue® Transfer Medium on Implantation of Human Embryos

Dragana Pavlović Vasić,¹ Sanja Sibinčić,¹ Irena Milaković,¹ Sanja Lukač,¹ Saša Vujnić,¹ Milica Gvero,¹ Danijela Madžar,¹ Aleksandra Govedarović,¹ Dragan Ivanović¹

Abstract

Introduction: Thanks to ever-growing advances in medical science, couples who are in the *in vitro* fertilisation (IVF) now have more options than ever to encase their chances at a successful pregnancy. One of the options is the use of EmbryoGlue (EG), that creates a bridge between the embryo and the uterus and provides protection to the embryo itself during the transfer process.

Aim of this study was to determine whether EG medium is of greater importance for embryo implantation than conventional medium in assisted reproductive technology and compare the rate of embryo implantation with EG and conventional medium in relation to the quality of the embryo, the age of the patients and tobacco smoking.

Methods: The retrospective study included 50 patients who used EG medium in embryo transfer (ET) and 50 patients in the control group using conventional medium. All patients underwent ET after stimulation of the cycle according to a short protocol. ETs were done on Day 2, 3, or 5 in the blastocyst stage. Age and smoking status were recorded.

Results: Out of a total of 100 patients, 42 patients had successful implantation and positive β -hCG 15 days after ET. In a control group 38 % had positive β -hCG and in the group of patients who used EG 46 %. A higher rate of embryo implantation success was observed on the second day of transfer in the group of patients using EG. In the EG group a significant increase in the embryo implantation rate was observed in patients older than 35. In tobacco smokers the implantation rate was higher if they used EG during ET.

Conclusion: EG medium had a positive effect on the second day of ET, patients above the age of 35 and patients who were tobacco smokers.

Key words: EmbryoGlue; Embryo; Hyaluronan; Implantation.

(1) Health institution Medico S, a member of the Pronatal group, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.

Correspondence:
DRAGANA PAVLOVIĆ VASIĆ
E: drdragana1972@gmail.com

ARTICLE INFO

Received: 7 April 2021
Revision received: 2 June 2021
Accepted: 4 June 2021

Introduction

Embryo transfer (ET) is a multi-stage process and one of the most sensitive steps in the *in vitro* fertilisation (IVF) procedure. From aspiration to transfer, it is necessary to support the oocytes and later, the development of the embryo by providing optimal cultivation conditions. The combination (ingredients) of the transfer medium is considered to be important for the interaction of the endometri-

um and the embryo during implantation.¹ After transfer, the embryo must rely on its own ability to implant and use of hyaluronan-enriched media can improve implantation success.

IVF programs continuously seek to increase the rate of implantation and pregnancies.² Advances in stimulation regimen and therapy, cultivation

media, and technology contribute to this.³ By designing cultivation media, which mimic changes in the female reproductive tract, it is possible to optimise development and create a healthy environment for the embryo.⁴

EmbryoGlue (Vitrolife)[®] (EG) is a medium modelled exclusively for ET with a proven effect of increasing implantation. EG has a basic composition of blastocyst culture medium and contains high concentrations of hyaluronan (HA) and recombinant albumin. It can be used to transfer all stages of embryonic development, including blastocysts after assisted hatching, biopsy, and cryopreservation. HA is a glycosaminoglycan naturally present *in vivo* in follicles, oviducts, uterine fluids and may be involved in the implantation process,^{5, 6} by binding to the CD44 glycoprotein receptor of the embryo allowing HA to enter the cell.^{7,8} HA is ubiquitous in the body, however, its action at different locations of the reproductive tract critically depends on its size, which is controlled by the balance of synthesis using one of the three isoforms.⁹

Due to its similar consistency, HA increases the viscosity of the transfer medium and may enhance the ability of the embryo to interact with uterine fluid. Thanks to these properties, it improves the apposition and binding of embryos, which are key steps in the implantation process.^{10, 11}

Aim of this study was to determine whether EG medium is of greater importance for embryo implantation than conventional medium in assisted reproductive technology (ART) and compare the rate of embryo implantation with EG and conventional medium in relation to the quality of the embryo, the age of the patients and tobacco smoking.

Material and methods

The retrospective study included 50 patients who used EG medium in ET and 50 patients in the control group using conventional medium. The period from June to November 2019 was analysed through available data from the history of ART Health Institution Medico S, a member of the Prenatal group. All patients underwent ET after stimulation of the cycle according to a short protocol. ETs were done on Day 2, 3, or 5 in the blastocyst stage. Age and smoking status were recorded.

Statistical processing was performed using the IBM SPSS software, version 15. All obtained data were processed by standard procedures of comparative statistics, Student's t-test and Chi-squared test, with statistical significance set as $p < 0.05$.

Results

a) Implantation success

Out of a total of 100 patients, 42 patients had successful implantation and positive beta human chorionic gonadotropin (β -hCG) 15 days after ET. In a control group (50 patients), 19 had positive β -hCG and 31 negative β -hCG, representing 38 % of embryo implantation success rate. In the group of patients who used EG, 23 patients had positive β -hCG and 27 negative β -hCG, which makes a 46 % success rate. There is an obvious difference in implantation success rate between these two groups of 8 % in favour of EG medium, however, difference was not statistically significant (Chi-squared test, $p = 0.418$) (Figure 1).

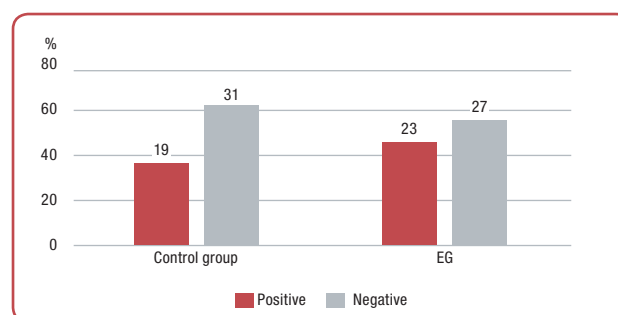


Figure 1: Embryo implantation success rate

*EG: EmbryoGlue %; percentage of total number of patients;

b) Quality of the embryo

In the control group, the success rate of embryo implantation (positive β -hCG) on the Day 2 was 28.6 % (4 of 14 patients), on the Day 3, 37 % (10 out of 27 patients) and on the Day 5, 55.6 % (5 out of 9 patients) (Figure 2).

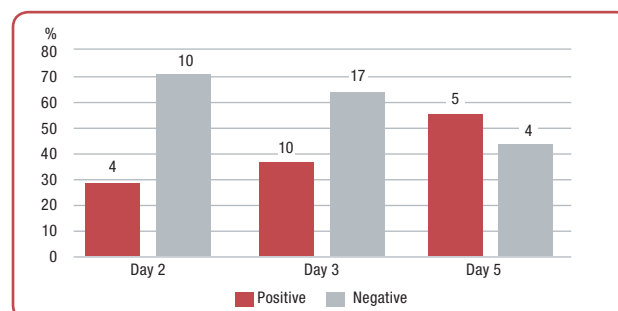


Figure 2: Implantation success in the control group

%: percentage of total number of patients;

In the group of patients who used EG medium, the success rate of embryo implantation (positive β -hCG) on the Day 2 was 56 % (9 out of 16 patients), on the Day 3, 45.8 % (11 out of 24 patients) and on the Day 5, 30 % (3 out of 10 patients) (Figure 3).

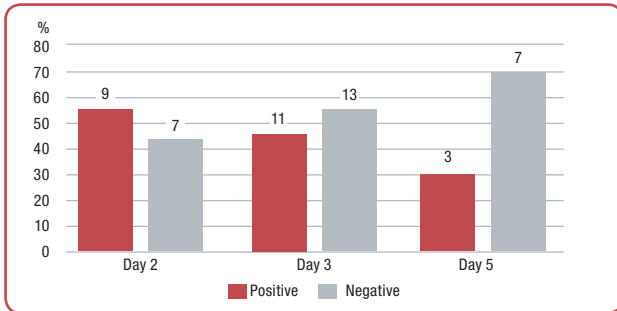


Figure 3: Success of implantation with EmbryoGlue medium %: percentage of total number of patients;

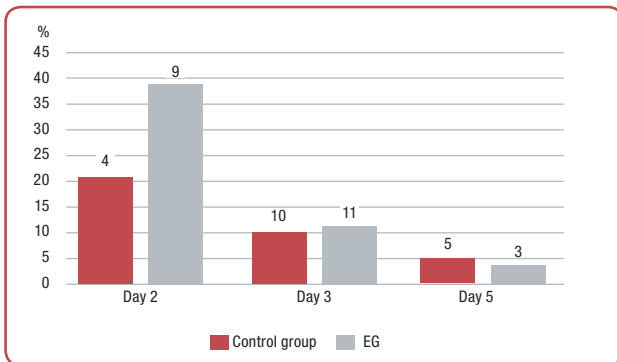


Figure 4: Comparison of implantation success depending on embryo quality between control and EmbryoGlue (EG) group %: percentage of total number of patients

Comparative analysis using Student t-test between these two groups did not show a statistical difference $p = 0.834$. However, a higher rate of embryo implantation success was observed on the second day of transfer in the group of patients using EG (Figure 4).

c) Influence of patient age

Patients were classified according to age into five groups (20-25, 26-30, 31-35, 36-40 and 41-45 years). At the age of 20-25, there were no patients with positive β -hCG hormone in the control and EG groups. In the age of 26-30, positive β -hCG was in 37.5 % patients (3 of 8 patients), while in the group where EG was used as a transfer medium, 20 % (1 of 5 patients). At the age of 31-35 years, there were 50 % positive β -hCG in the group with EG medium (8 of 16 patients), while in the control group it was 43.5 % (10 of 23 patients). Of the number of patients aged 36-40 years, 48 % had positive β -hCG who used EG medium (12 of 25 patients), while in the control group it was 37.5 % (6 of 16 patients). At the age

of 41-45, there were 66.7 % of patients with positive β -hCG in the group with EG medium (2 of 3 patients), and there were no implantation in the control group (Figure 5).

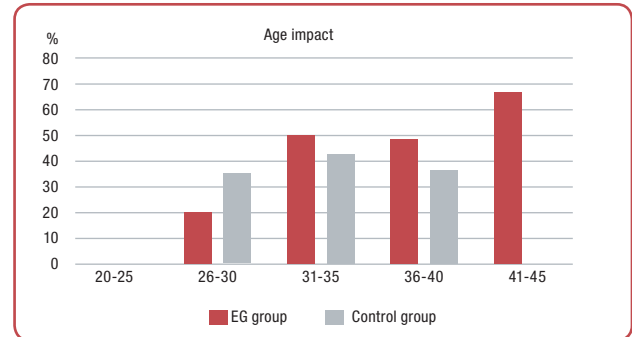


Figure 5: Comparison of implantation success between EmbryoGlue (EG) and control group in relation to age %: percentage of total number of patients;

Student-t test did not reveal a significant difference in age between these two groups ($p = 0.182$). By analysing the success rate of the implantation rate within the age groups, in the EG group a significant increase in the embryo implantation rate was observed in patients older than 35 years.

d) Impact of tobacco smoking

Impact of tobacco smoking on success in the implantation of embryos was analysed. In the group with EG medium, there were 32 % of smokers and 68 % of non-smokers, and in the control group there were 38 % smokers and 62 % non-smokers.

Table 1: Impact of tobacco smoking on implantation success

Tobacco smokers	Control group		EmbryoGlue group	
	Total	Positive β -hCG	Total	Positive β -hCG
	N	%	N	%
Yes	19	38	4	21.1
No	31	62	15	48.3
	34	68	16	47.1

* β -hCG: β -human chorionic gonadotropin

Embryo implantation (positive β -hCG) in the group of patients who used EG medium in smokers was 43.7 %, while in non-smokers it was 47.1 % (Table 1). In the control group of female patients, 21.1 % of smokers had positive β -hCG, and in the non-smoking group, β -hCG was positive in 48.3 %. Due to the small number of samples in the observed parameters, difference is not statistically significant, but it is noticed that in smokers the implantation rate was higher if they used EG during ET.

Discussion

As already mentioned, EG contains HA and nutrients that are necessary to support embryos from transfer to the moment of implantation as well as for the process of biochemical interaction during that period. The positive effect of EG medium was proven by its introduction on the market in 2003. After that, several studies published its positive effect. In 2010, an independent Cochrane Collaboration published a meta-analysis of controlled randomised studies "ET medium adhesion components".¹ Using EG for ET, the rate of clinical pregnancies increased significantly from 41 % to 50 % compared to conventional media with low or even no HA content (meta-analysis of 13 publications covering Cochrane articles in more than 3,200 patients). Analyses did not show an increase in abortions or other unforeseen circumstances (outcomes). Cochrane authors identified a clear positive effect of high concentrations of HA in transfer media. Their report published in 2020 shows an increased pregnancy rate after using high concentrations of HA in ET media. Results from this study confirm the previous conclusions of the Cochrane report. In this study, a positive effect of EG medium was proven, since the implantation rate is higher after its use, although there is no statistically significant difference in the observed parameters.

Numerous clinics in Japan that use EG transfer medium presented different results at scientific conferences between 2010 and 2013. Meta-analyses of Japanese studies involving nearly 10,000 cycles show increased implantation and pregnancy rates from fresh and cryo transfers, after the use of EG medium.^{12, 13} Their results confirm the previous conclusions of the Cochrane report. HA-enriched media may increase the implantation rate¹⁴ and clinical pregnancies in patients with previous failed implants and in the third or more attempts after cryo ET.^{10, 15} They also report that clinical pregnancies and implantation rates were higher in embryos after cryo ET, indicating that the viscosity of the EG medium physically protects embryos after assisted hatching.¹³

Zhang et al¹⁶ and Wu et al¹⁷ investigated whether EG has an impact on pregnancy outcome during *in vitro* fertilisation compared to G2 medium. In conclusion, they cite EG as a transfer medium that can improve the rate of implantation and pregnancy in the IVF program. Based on their results, there is no statistically significant difference in the

age of patients, duration of infertility, number of failed cycles, endometrial thickness, number and between embryo quality and the average number of embryos in the observed groups. The results of this study show that the level of implantation is more successful in women above 35 years of age who used EG, while in women younger than 35 the rate of implantation in both groups is approximately the same. Urman et al confirm the beneficial effect of HA-enriched medium, which is evident in women over 35 years of age, in women with poorer embryo quality and with previous unsuccessful implantation.⁶

Svobodová et al¹⁸ observed a statistically significant beneficial effect of EG medium on embryo implantation in patients aged 30-38 years. This research confirms the positive effect of EG medium on Day 2 of transfer, which shows that embryos in the early phase of transfer have a higher chance of more successful implantation with this medium, while for transfers 3 and 5 days the results are similar between the control and EG groups. Urman et al found that HA-enriched media increased the rate of clinical pregnancies and implantation on days 3 and 5 of ET.⁶

Several authors state that HA-enriched media have not shown significant results in their study^{2, 19} and concluded that future research should show whether EG is the highest quality option for all patients or only for those patients in whom the quality of the embryo is poor, for patients in whom implantation has failed and older patients.²⁰ Nishihara et al²¹ state that pregnancies, implantation, and abortion rates where media with different concentrations of HA was used during ET gives similar results.

Toxic components of cigarette smoke affect the gametes, ovarian, tubal, endometrial and myometrial components. Uterine receptivity is also impaired.²²

When comparison was made on the effect of EG on embryo implantation in smoking and non-smoking patients, conclusion was made that support the justified use of EG in women who smoke, because the rate of embryo implantation in women smokers who used EG as a medium is significantly higher than those who used a conventional medium. In non-smoking women there was no large difference in embryo implantation between these two media. The sample was not large enough to have a statistically significant difference.

Conclusion

According to the results obtained, conclusion could be made that there is a difference in the success rate of implantation with EG medium. A higher rate of embryo implantation on the second day of transfer and in older patients (> 35 years) with EG medium was observed, although the statistical analysis did not reveal a significant difference in these categories.

In smokers, the rate of implantation with EG medium was higher compared to the conventional medium, while in non-smokers there was no difference.

EG medium had a positive effect on the second day of ET, patients above the age of 35 and patients who were tobacco smokers.

Acknowledgements

None.

Conflict of interest

None.

References

- Bontekoe S, Blake D, Heineman MJ, Williams EC, Johnson N. Adherence compounds in embryo transfer media for assisted reproductive technologies. *Cochrane Database Syst Rev* 2010 Jul 7;(7):CD007421.
- Hazlett WD, Meyer LR, Nasta TE, Mangan PA, Karande VC. Impact of EmbryoGlue as the embryo transfer medium. *Fertil Steril* 2008;90:214-6.
- Gardner D, Lane M. Development of viable mammalian embryos in vitro: Evolution of sequential media. In: Cibelli, J, Lanza, R, editors. *Principles of Cloning*. 1st ed. Cambridge, MA, USA: Academic Press; 2002. p. 187-213.
- Chronopoulou E, Harper JC. IVF culture media: past, present and future. *Hum Reprod Update* 2015 Jan-Feb;21(1):39-55.
- Nagyova E. The biological role of hyaluronan-rich oocyte-cumulus extracellular matrix in female reproduction. *Int J Mol Sci* 2018 Jan 18;19(1):283.
- Urman B, Yakin K, Ata B, Isiklar A, Balaban B. Effect of hyaluronan-enriched transfer medium on implantation and pregnancy rates after day 3 and day 5 embryo transfers: a prospective randomized study. *Fertil Steril* 2008 Sep;90(3):604-12.
- Berneau SC, Ruane PT, Brison DR, Kimber SJ, Westwood M, Aplin JD. Investigating the role of CD44 and hyaluronate in embryo-epithelial interaction using an in vitro model. *Mol Hum Reprod* 2019 May 1;25(5):265-273.
- Campbell S, Swann HR, Aplin JD, Seif MW, Kimber SJ, Elstein M. CD44 is expressed throughout pre-implantation human embryo development. *Hum Reprod* 1995 Feb;10(2):425-30.
- Fouladi-Nashta AA, Raheem KA, Marei WF, Ghafari F, Hartshorne GM. Regulation and roles of the hyaluronan system in mammalian reproduction. *Reproduction* 2017 Feb;153(2):R43-R58.
- Singh N, Gupta M, Kriplani A, Vanamail P. Role of Embryo Glue as a transfer medium in the outcome of fresh non-donor in-vitro fertilization cycles. *J Hum Reprod Sci* 2015 Oct-Dec;8(4):214-7.
- Schoolcraft W, Lane M, Stevens J, Gardner DK. Increased hyaluronan concentrations in the embryo transfer medium results in a significant increase in human embryo implantation rate. *FertilSteril* 2002 Sep;78(Suppl 1):S5.
- Hashimoto S, Joris H, Morimoto Y. EmbryoGlue® improves pregnancy and implantation rates: results from a meta-analysis on almost 10,000 embryo transfers. *Reprod Biomed Online* 2014;28(1):S7-S8.
- Tomari H, Honjou K, Kunitake K, Nishimura K, Hidaka N, Nagata Y. Effect of embryo glue transfer medium during fresh and frozen-thawed embryo transfer. *Fert Ster* 2014 Oct;102(3):e313.
- Zbořilová B, Oborná I, Tkadlec E, Procházka M, Březinová J, Sobek A Jr, et al. Does EmbryoGlue transfer medium affect embryo transfer success rate? *Ceska Gynekol* 2018 Winter;83(3):177-81.
- Fu W, Yu M, Zhang XJ. Effect of hyaluronic acid-enriched transfer medium on frozen-thawed embryo transfer outcomes. *J Obstet Gynaecol Res* 2018 Apr;44(4):747-55.
- Zhang JR, Guan YC, Zhang LW, Hao DY, Wang XM, Liu J, et al. Influence of transfer medium EmbryoGlue on clinical outcome of embryo transfer. *Shanghai Jiao Tong Da Xue Xue Bao Yi Xue Ban* 2013;33(1):71-4.
- Wu F, Lü R, Bai XH, Song XR. [Influence of EmbryoGlue on the implantation of embryo and pregnancy outcome in vitro fertilization-embryo transfer]. *Zhonghua Fu Chan Ke Za Zhi* 2012 Feb;47(2):121-4. Chinese.
- Svobodová M, Březinová J, Oborná I, Dostál J, Krsková M. Transferové médium s hyaluronanem embryoGlue v programu IVF+ET [EmbryoGlue the transfer medium with hyaluronan in the IVF+ET program]. *Ceska Gynekol* 2007 Jan;72(1):15-9. Czech.
- Chao S, Schenkman E, Kim S, Kenigsberg D, Brenner S, Moodie G. The effect of embryo glue on clinical pregnancy rate in frozen embryo transfers. *Fertil Steril* 2008;90:S434.
- Safari S, Razi MH, Safari S, Razi Y. Routine use of Embryo-Glue(®) as embryo transfer medium does not improve the ART outcomes. *Arch Gynecol Obstet* 2015 Feb;291(2):433-7.
- Nishihara T, Morimoto Y. Evaluation of transfer media containing different concentrations of hyaluronan for human in vitro fertilization. *Reprod Med Biol* 2017 Aug 18;16(4):349-53.
- Klonoff-Cohen H, Natarajan L, Marrs R, Yee B. Effects of female and male smoking on success rates of IVF and gamete intra-Fallopian transfer. *Hum Reprod* 2001 Jul;16(7):1382-90.