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Routine use of EmbryoGlue® as embryo transfer medium does not improve the ART outcomes

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Abstract
Purpose The aim was to investigate assisted reproductive technology (ART) outcomes after routine use of EmbryoGlue® as a human embryo transfer (ET) medium with high concentration of hyaluronan (HA, hyaluronic acid).
Methods A cohort of total 229 patients was retrospectively enrolled for the present study. They were subjected for embryo transfer on day 2 either in EmbryoGlue® ($n = 117$) as study group or in conventional ET medium with low concentration of HA as control group ($n = 112$).
Results Patients in the both groups, in regards to the mean level of day 3 FSH, the etiology of infertility, the history of implantation failure and the rate of good quality embryos showed similar characteristics. There were no significant differences between two groups in terms of clinical and ongoing pregnancies, implantation, delivery and live birth rates. In spite of a decreased abortion and increased multiple pregnancy rates in the study group compared to the control group (15.8 vs. 19 % and 20.6 vs. 15.6 respectively), the differences were not statistically significant.
Conclusions Routine use of EmbryoGlue®as a HA enriched ET medium for cleavage stage embryos does not have advantage to the conventional one for infertile patients undergoing ART.

Keywords Hyaluronan · EmbryoGlue® · Embryo transfer · Pregnancy outcome

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Introduction

The ART success rates have been significantly improved over last decades; however, embryo implantation still remains a major limiting factor [1]. It has been considered that composition of an embryo transfer medium is important for interaction between embryo and endometrium at the time of implantation [2]. Nevertheless, the role and composition of ET medium have not been investigated widely [3]. Moreover, essential factors that appear to be contributed to embryo development and implantation have not been yet determined entirely [4]. As more is known about the in vivo conditions that an embryo is exposed, some modifications to the embryo culture media are developed to mimic the original environment. One of the examples for that is supplementation of ET media with HA, a major glycosaminoglycan in uterine fluid, which assumed to improve the process of implantation [5].

Several properties of HA that makes it a potential candidate as an implantation-enhancing component have been elucidated [2]; however, the mechanism by which HA promotes implantation has not yet been clarified. It has been shown that HA increases cell–cell and cell–matrix adhesion, which may function during embryo apposition, attachment and implantation. Both the endometrium of uterine and embryo express receptors for HA. This binding of HA to the embryo might facilitate its attachment to the uterine endometrium until the embryo grows to a hatched blastocyst [1]. Indeed, HA produce a viscous environment that might enhance ET process, while inhibiting the expulsion of embryos from the uterine cavity after ET [6]. In addition, some degree of viral protection and anti-immunogenic properties are other characteristics of HA which prohibit the rejection of an embryo from the uterine cavity [7, 8]. Based on these data, HA-enriched transfer

medium (HETM) became a possible candidate for improving implantation process [1]. Therefore, EmbryoGlue® as a HETM was introduced to support embryos at the time of ET for promoting the implantation process [5].

Some clinical reports showed improvement in both pregnancy and implantation rates with HETM; while, others have reported no beneficial effects [1]. Therefore, the aim of this study was to evaluate whether routine use of EmbryoGlue® as a HETM would be beneficial for improvement of outcomes in an unselected group of patients undergoing ART program.

Materials and methods

Patients

A cohort of total 229 unselected patients that underwent for ART treatment, at Research and Clinical Center for Infertility, Yazd, Iran, from May 2011 to December 2012 were retrospectively enrolled in this study. For the study group ($n = 117$), the ET medium was EmbryoGlue® (Vitrolife, Sweden) containing recombinant human albumin and a high concentration of recombinant HA (rHA). For the control group ($n = 112$), the ET medium was G-2^{TM}v5 (Vitrolife, Sweden) containing HSA and a lower concentration of rHA. The study was approved by ethics committee and institutional review board. All the patients signed informed consents.

Ovarian hyperstimulation, ICSI and IVF procedures

Controlled ovarian hyperstimulation was performed following pituitary down-regulation as described previously [9]. Oocyte retrieval was performed approximately 36 h after hCG administration by transvaginal ultrasound-guidance. All sperm preparations were performed with density gradient centrifugation or swim-up techniques [10, 11]. Conventional IVF or ICSI procedures were applied according to etiology of infertility [12, 13].

Fertilization and embryo evaluation

After ICSI or denudation after conventional IVF, the oocytes were washed twice and were placed in micro droplets of G-1^{TM}v5 medium (20 µl) (Vitrolife, Sweden) covered with mineral oil. After 16–18 h of incubation, the oocytes were assessed for presence of two pronuclei (2PN). The zygotes were kept in G-1^{TM}v5 until ET on day 2. About 48 h post-injection or insemination, the embryos were morphologically classified according to Hill et al. [14] as following: Grades A: Even blastomeres with no fragmentation. B: A little inequality in blastomeres size, <10 % cytoplasmic fragments. C: Unequal sized blastomeres, <50 % fragments. D: Unequal blastomeres, severe fragmentation and large black granules. Grades A and B were considered as high quality embryos. Grade D embryos were discarded.

Embryo transfer and luteal support

In the control group, embryos with grade A, B and C were transferred into G-2^{TM}v5 medium. However, in the study group, they were transferred into EmbryoGlue®. These media were preincubated overnight in an environment of 5 % O_2, 6 % CO_2 at 37 °C. The catheters (Cook; Cook Medical, USA) were loaded by an embryologist and handed to a clinician for performing ET.

In agonist protocol, the luteal phase was supported by intravaginal progesterone (Progesterone, Aburaihan Co., Iran) 400 mg BID, starting from the day of oocyte retrieval until the 10th week of gestation. Also, in antagonist protocol for luteal support patients received estradiol (Aburaihan Co., Iran) 2 mg BID, in addition to progesterone.

Chemical pregnancy was confirmed by measuring βhCG level on day-14 of ET. Clinical pregnancy was defined as presence of fetal heartbeat that was evaluated seven weeks after ET using ultrasound. Implantation rate was calculated as total number of intrauterine gestational sacs divided by total number of transferred embryos multiplied by 100. Ongoing pregnancy was defined as pregnancy proceeding beyond the 12th week of gestation. Abortion rate was defined as number of clinical pregnancy losses before 20th week of gestation divided by the total of chemical pregnancy. Delivery rate was defined as a ratio between deliveries and ET cycles. Live birth rate was defined as a ratio between number of healthy newborns and number of ET cycles.

Statistical analysis

Distribution of data was analyzed by Kolmogorov–Smirnov test and accordingly Mann–Whitney U test, Chi-square test and independent samples t test applied for the analysis. $P < 0.05$ was considered statistically significant. Data analysis was performed with SPSS 17 software.

Results

Causes of infertility in the study and the control groups are summarized in Table 1. The patients' characteristics in the aforementioned groups are presented in Table 2. There were no significant differences between the groups in regard to the number of IVF or ICSI cycles, patient's ages, FSH level on day 3, the number of patients without

Table 1 Causes of infertility of patients who were included in the study

	Study group (%)	Control group (%)	*P* value
Male factor	72 (63.2)	67 (60.9)	0.78
Ovarian dysfunction	17(14.9)	16 (14.5)	1.00
Endometriosis	8 (7.0)	4 (3.6)	0.37
Tubal factor	6 (5.3)	10 (9.1)	0.30
Multiple factors	7 (6.1)	6 (5.5)	1.00
Unexplained	4 (3.5)	7 (6.4)	0.37

previous failed cycles, the number of retrieved oocytes or metaphase II oocytes and fertilized oocytes. Likewise, the cycles with high quality embryos and the number of transferred embryos were similar between the two groups. The rates of clinical and ongoing pregnancies, implantation, delivery and live birth in the study group were similar when compared with the control group (Table 3). In the study group, the rate of multiple pregnancies was numerically higher than those in the control group (20.6 vs. 15.6 %, respectively).

In the study group, seven twin pregnancies occurred among the 34 clinical pregnancies compared to three twins and two triplets out of 32 pregnancies in the control group. The differences in twin pregnancies between two groups were insignificant (20.6 vs. 9.4 %, respectively). However, the difference in triplet pregnancies between two groups was significant (0 vs. 6 %, respectively). The comparison of the abortion rate between the study (15.8 %) and the control group (19 %) was not significant.

Discussion

The beneficial effect of HA in transfer medium is not yet studied well and remained as a controversial issue.

Table 3 Comparison of clinical outcome between study (EmbryoGlue®) and control (G-2™v5) groups

Parameter	Study group ($n = 117$)	Control group ($n = 112$)	*P* value
Chemical pregnancy rate	38/117 (32.5)	37/112 (33)	0.92
Clinical pregnancy rate	34/117 (29.1)	32/112 (28.6)	1.00
Singleton	27/34 (79.4)	27/32 (84.4)	0.75
Multiple pregnancy	7/34 (20.6)	5/32 (15.6)	0.75
Twin	7/34 (20.6)	3/32 (9.4)	0.3
Triplet	0/34 (0)	2/32 (6.2)	0.009[a]
Implantation rate	41/267 (15.4)	42/244 (17.2)	0.63
Ongoing pregnancy rate	32/117 (27.4)	31/112 (27.7)	1.00
Abortion rate	6/38 (15.8)	7/37 (19)	0.74
Delivery rate	30/117 (25.6)	27/112 (24.1)	0.88
Live birth rate	37/117 (31.6)	35/112 (31.3)	1.00

Values in parentheses are percentage

[a] Statistic is significant at 0.05

Hambiliki et al. [15] reported that both embryo and endometrium produce HA. Therefore, they reasoned that there is no need to add HA into the ET medium. Several studies have revealed that HA- enriched medium can improve implantation and pregnancy rates [3, 16, 17]. On the other hand, it has been shown that HA in transfer media does not improve implantation rates [4, 8, 18, 19]. Our findings support the later studies, verifying that the beneficial effect of EmbryoGlue® is inconclusive. Our observation showed no significant differences in pregnancy, implantation and ongoing pregnancy rates between both studied ET media.

In the present study, the same number of selected embryos was transferred in both of the study and the control groups; however, multiple pregnancy rate in the study group was 5 % higher than the control group (20.6

Table 2 Comparison of patients' characteristics between study (EmbryoGlue®) and control (G-2™v5) groups

Parameter	Study group ($n = 117$)	Control group ($n = 112$)	*P* value
IVF/ICSI cycles (%)	9/108 (8.3)	12/100 (12)	0.40
Females' age (Mean ± SD)	30.33 ± 5.43	30.15 ± 5.32	0.79
Day 3 FSH, mIU/ml (Mean ± SD)	6.96 ± 3.06	6.6921 ± 3.43	0.56
Patient without previous failed cycles (%)	94 (81.7)	91 (82.7)	0.86
No. of oocytes retrieved median (minimum–maximum)	9 (1–25)	10 (1–33)	0.94
No. of MII oocytes median (minimum–maximum)	7 (1–22)	7 (1–23)	0.96
No. of fertilized oocytes median (minimum–maximum)	4 (1–18)	3.5 (1–15)	0.07
Cycles with high quality embryos (%)	97/117 (83)	96/112 (86)	0.6
No. of transferred embryos median (minimum–maximum)	2 (1–4)	2 (1–4)	0.31

vs. 15.6 %). In addition, twin pregnancies were higher in the study group (20.6 vs. 9.4 %, respectively). These outcomes are in agreement with those achieved by Simon et al. [18], Valojerdi et al. [8] and Friedler et al. [16] studies in which multiple pregnancy in HETM group was insignificantly higher. Whereas, in Urman's study, this difference was significantly higher [3]. Increase in multiple pregnancies may be the result of improved pregnancy outcomes with HA along with transferring of more than one embryo [2]. Therefore, reducing the number of transferred embryos should be considered to avoid multiple pregnancies if HETM is used [3]. Interestingly, the triplet pregnancies in the control group were slightly higher when compared to the study group (6 vs. 0 %). The abortion rate was insignificantly decreased in women who received high concentration of HA (15.8 vs. 19 %). These results were in line with the previous reports [3, 16], confirming the abortion rate is not influenced by high concentration of HA.

Another finding in our study was to identify the delivery rate. In the study group, 30 women have given birth (37 healthy newborns) for a take-home baby rate of 31.6 % that was comparable to the control group. In previous studies, the live birth rate after using HETM was infrequently reported and no conclusions could be made [2]. As discussed here, our results were sometimes in agreement or in contrast with the previous studies. This could be explained by the fact that the patient inclusion criteria varied considerably among these studies.

It is notable that our unselected patient's cohort was relatively young with mean level of FSH <7 and the majority of patients had no previous unsuccessful ETs. Their response to the controlled ovarian stimulation was satisfactory and median of seven mature oocytes retrieved, which led to the development of high quality embryos. The rates of high quality transferred embryos in both study and control groups were high. Considering all the mentioned data, these patients showed good prognosis during ART treatment. It seems that using HETM for patients with good prognosis has no beneficial effect on pregnancy outcomes. In agreement with our findings, the previous studies that used high concentration of HA for ET in selected good prognosis patients (with limited previous embryo transfer attempts, female age ≤35, having good quality embryos) [5, 18, 20], showed no beneficial effect on clinical pregnancy, ongoing pregnancy and implantation rates as well. However, studies which are done on poor prognosis cohort [16] were indicating that HETM improved all the aforementioned parameters.

Our findings do not support routine use of EmbryoGlue® for all patients seeking ART treatment program. Loutradi et al. [4] showed no improvement in clinical pregnancy rates in a non-selected group of patients using EmbryoGlue® compared to G-2™v3. They stated that a high concentration of HA in ET medium in the presence of recombinant HSA neither compromised nor improved the pregnancy rates. The other study by Valojerdi et al. [8] in a prospective randomized study showed that when considering all patients together, no differences were found regarding implantation or clinical pregnancy rates between the study and control groups. In contrast, the study conducted by Friedler et al. [16] indicated that in patients with at least four previous unsuccessful transfer, the use of a commercially available ET medium enriched with HA (EmbryoGlue®) caused significant improvement in implantation and in clinical pregnancy rates.

The beneficial effect of HETM was obvious, especially in women with recurrent implantation failure (RIF), in women >35 years old, and in cases that received poor-quality embryos. Increased implantation rate in women with poor quality embryos and in older women is in regard to this hypothesis that, HETM increases the potential of embryos implantation. A noticeable increase in implantation and multiple pregnancy rates using HETM compared to the clinical pregnancy rate also supports this hypothesis. A woman's age can affect oocyte and embryo quality, rather than endometrium. The utilization of HETM in this group of patients most likely increases implantation by its effects on embryo [3]. Nakagawa et al. [1] reported that inadequate levels of HA might explain some of patients' history of four or more unsuccessful ETs. Therefore, the improvement of embryo implantation in poor prognosis patients could be due the compensation level of HA used in the transfer medium.

The possible reason that using HA did not show any effect on implantation rate in most of the previous studies is that HA was present in both the study and the control transfer media [16]. The differences between EmbryoGlue® and G-2™v5 were in increasing concentration of HA by fourfold and in reduction of the recombinant human albumin by fourfold in EmbryoGlue® [3]. Likely, the difference in HA concentration was not great enough to cause a significant rise in implantation rate, particularly in a non-selected group of patients [16].

It should be noted that, in most previous prospective studies [1, 3, 5], delivery and live birth rates after using HETM were poorly reported and a comprehensive conclusions could not be made [2]. Therefore, despite the limitation of our study that was retrospective, we reported delivery and live birth rates in addition to clinical, ongoing and multiple pregnancies, implantation and abortion rates in the unselected cohort of patients.

In conclusion, our data showed that high concentration of HA in ET medium neither compromised nor improved ART outcomes. In spite of the limitations of the current study, it is important to report that EmbryoGlue® may not be the best option for all patients. Therefore, further

prospective double-blind controlled trials would be better option for: (1) to make net conclusions regarding the role of HA as an implantation enhancing factor; (2) to determine whether EmbryoGlue® is the best option in a selected patient population, such as patients with poor quality embryos, RIF and advanced maternal age.

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References

1. Nakagawa K, Takahashi C, Nishi Y, Jyuen H, Sugiyama R, Kuribayashi Y (2012) Hyaluronan-enriched transfer medium improves outcome in patients with multiple embryo transfer failures. J Assist Reprod Genet 29(7):679–685. doi:10.1007/s10815-012-9758-2
2. Bontekoe S, Blake D, Heineman MJ, Williams EC, Johnson N (2010) Adherence compounds in embryo transfer media for assisted reproductive technologies. Cochrane Database Syst Rev (7): CD007421. doi:10.1002/14651858.CD007421.pub2
3. Urman B, Yakin K, Ata B, Isiklar A, Balaban B (2008) 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 90(3):604–612
4. Loutradi KE, Prassas I, Bili E, Sanopoulou T, Bontis I, Tarlatzis BC (2007) Evaluation of a transfer medium containing high concentration of hyaluronan in human in vitro fertilization. Fertil Steril 87(1):48–52
5. Hazlett WD, Meyer LR, Nasta TE, Mangan PA, Karande VC (2008) Impact of EmbryoGlue as the embryo transfer medium. Fertil Steril 90(1):214–216
6. Gardner DK, Rodriegez-Martinez H, Lane M (1999) Fetal development after transfer is increased by replacing protein with the glycosaminoglycan hyaluronan for mouse embryo culture and transfer. Hum Reprod 14(10):2575–2580
7. Laurent TC, Fraser JRE (1992) Hyaluronan. FASEB J 6(7):2397–2404
8. Valojerdi MR, Karimian L, Yazdi PE, Gilani MA, Madani T, Baghestani AR (2006) Efficacy of a human embryo transfer medium: a prospective, randomized clinical trial study. J Assist Reprod Genet 23(5):207–212. doi:10.1007/s10815-006-9031-7
9. Halvaei I, Khalili MA, Razi MH, Agha-Rahimi A, Nottola SA (2013) Impact of different embryo loading techniques on pregnancy rates in in vitro fertilization/embryo transfer cycles. J Hum Reprod Sci 6(1):65–69. doi:10.4103/0974-1208.112385
10. Khalili MA, Razavi V, Mardanian F, Esfandiari N (2008) The predictive value of pronuclear morphology screening on embryo development and pregnancy outcome in ART cycles. Middle East Fertil Soc J 13(1):44–51
11. Khalili MA, Mir-Rokni F, Kalanter SM (1999) Application of vitality tests on asthenozoospermic semen from infertile men. Iran Biomed J 3:77–81
12. Halvaei I, Khalili MA, Soleimani M, Razi MH (2011) Evaluating the role of first polar body morphology on rates of fertilization and embryo development in ICSI cycles. Int J Fertil Steril 5(2):110–115
13. Khalili MA, Mojibian M, Sultan AM (2005) Role of oocyte morphology on fertilization and embryo formation in assisted reproductive techniques. Middle East Fertil Soc J 10:72–77
14. Hill G, Freeman M, Bastias M, Rogers B, Herbert C 3rd, Osteen K, Wentz A (1989) The influence of oocyte maturity and embryo quality on pregnancy rate in a program for in vitro fertilization-embryo transfer. Fertil Steril 52(5):801–806
15. Hambiliki F, Ljunger E, Karlström P-O, Stavreus-Evers A (2010) Hyaluronan-enriched transfer medium in cleavage-stage frozen-thawed embryo transfers increases implantation rate without improvement of delivery rate. Fertil Steril 94(5):1669–1673
16. Friedler S, Schachter M, Strassburger D, Esther K, El RR, Raziel A (2007) A randomized clinical trial comparing recombinant hyaluronan/recombinant albumin versus human tubal fluid for cleavage stage embryo transfer in patients with multiple IVF-embryo transfer failure. Hum Reprod 22(9):2444–2448
17. Korosec S, Virant-Klun I, Tomazevic T, Zech NH, Meden-Vrtovec H (2007) Single fresh and frozen–thawed blastocyst transfer using hyaluronan-rich transfer medium. Reprod Biomed Online 15(6):701–707
18. Simon A, Safran A, Revel A, Aizenman E, Reubinoff B, Porat-Katz A, Lewin A, Laufer N (2003) Hyaluronic acid can successfully replace albumin as the sole macromolecule in a human embryo transfer medium. Fertil Steril 79(6):1434–1438
19. Ravhon A, Nahum H, Weissman A, Biran G, Umansky N, Levran D (2005) Embryo transfer in hyaluronan enriched transfer medium does not improve pregnancy rate in IVF treatment. Fertil Steril 84:S376–S377
20. Mahani I, Davar R (2007) Hyaluronic acid versus albumin in human embryo transfer medium. East Mediterr Health J 13(4):876–880