Hyaluronic acid versus albumin in human embryo transfer medium

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المقارنة بين حمض الهيالورونيك والألبومين في وسط نقل الجنين البشري

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الخلاصة: قارنت الباحثتان معدلات الحمل والتعشيش في الإخصاب المختبري IVF باستخدام وسط ناقل أساسُه حمض الهيالورونيك مع وسط آخر أساسُه الألبومين، لدى ستين امرأة وُزِّعن توزيعاً عشوائياً إلى مجموعتَيْن؛ نقلت فيها الأجنَّة في المجموعة A وهي مجموعة المعالجة وتضم 30 حالة إلى أوساط أساسُها حمض الهيالورونيك، في حين نقلت الأجنَّة في المجموعة الثانية B وهي مجموعة الشواهد وتضم 30 حالة إلى أوساط أساسُها الألبومين. و لم يُلاحَظ أيُّ فرق يُعْتَدُّ به إحصائياً بين المجموعتَيْن من حيث العمر الوسطي للنساء والفترة الوسطية للعقم والعدد الوسطي للأجنَّة. وقد كان معدل الحمل في المجموعة A 81.8٪، وفي المجموعة B 71.4٪، وهو اختلاف لا يُعْتَدُّ به إحصائياً، ممَّا يدلُّ على إمكان الاستعاضة عن الألبومين بحمض الهيالورونيك بنجاح.

ABSTRACT We compared the implantation and pregnancy rate through *in vitro* fertilization (IVF) using hyaluronic acid and albumin as transfer medium in 60 women randomly allocated to 2 groups. In treatment group A (*n* = 30), embryos were transferred to medium supplemented with hyaluronic acid. In the control group B (*n* = 30), embryos were transferred to medium containing albumin. There were no significant differences between the groups in terms of mean age of the females, mean duration of infertility and mean number of embryos. The pregnancy rate in groups A and B were 81.8% and 71.4% respectively, a non-statistically significant difference. Hyaluronic acid can successfully replace albumin as transfer medium.

Acide hyaluronique *versus* albumine dans le milieu de transfert d'embryon humain

RÉSUMÉ Nous avons comparé chez 60 femmes randomisées en 2 groupes les taux d'implantation embryonnaire (ou nidation) et de grossesse obtenus par une technique de fécondation *in vitro* (FIV) utilisant un milieu de transfert supplémenté soit en acide hyaluronique (groupe thérapeutique A, n = 30), soit en albumine (groupe témoin B, n = 30). Il n'est apparu aucune différence significative entre les groupes en ce qui concerne l'âge moyen des femmes, l'ancienneté moyenne de la stérilité et le nombre moyen d'embryons. Le taux de grossesse a été respectivement de 81,8 % et 71,4 % dans les groupes A et B, soit une différence non statistiquement significative. L'acide hyaluronique peut se substituer avec succès à l'albumine comme milieu de transfert.

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Introduction

From the early beginnings of *in vitro* fertilization (IVF) it has been recognized that the culture media supplemented with proteins have a direct role in osmoregulation. Proteins also serve as a source of energy and as reservoirs for the release of hormones, vitamins and minerals [*1*]. Because of its characteristic high abundance in the female reproductive tract, albumin has traditionally served as the main macromolecule in most culture media used for *in vitro* growth of human embryos [*2*]. In addition, albumin confers on the culture medium the useful physical properties of lubrication and viscosity, thus promoting ease of handling the embryo and preventing its adherence to the culture dish [*3*].

Because the use of blood-derived albumin can cause both biological variation and the possibility of disease transmission, several macromolecules, such as polyvinylpyrolidone, polyvinyl alcohol and hyaluronic acid have been suggested as alternatives [*4*]. Although the potential of polyvinylpyrolidone and polyvinyl alcohol macromolecules to promote efficient embryo development *in vitro* is still questionable [*5,6*], hyaluronic acid effectively supports mouse and human embryo development and also their growth [*7,8*].

Hyaluronic acid is a naturally existing macromolecule related to the glycosaminoglycans family extra-cellular matrix and it is a linear polysaccharide [*9*]. It has been observed that both human [*10*] and bovine [*11*] embryos possess a surface receptor for hyaluronic acid that can be detected throughout their development up to the blastocyst stage. Research on bovine embryos suggests that the use of hyaluronic acid in the culture medium can increase the rate of bovine blastocyst and embryo development in IVF programmes [*12*]. Furthermore, hyaluronic acid, when added to sperm preparation media, increases sperm motility and improves retention of sperm motility in long-term incubation of both fresh and cryopreserved, thawed human spermatozoa [*13*]

Because there have been relatively few human studies with hyaluronic acid and because we have not made use of this medium before in our department, we aimed to compare the IVF implantation and pregnancy rate by using hyaluronic acid and albumin as transfer medium.

Methods

The study design is a prospective double-blind study performed from September 2003 to January 2004. As the embryos were grown at the embryonic laboratory, the doctors and women were unaware which medium had been used. It was conducted at the Research and Clinical Centre for Infertility in Yazd, Islamic Republic of Iran and was approved by the local ethics committee.

The study included 60 women undergoing IVF who were divided into Group A (whose embryos were transferred to a medium supplemented with hyaluronic acid) and a control Group B (whose embryos were transferred to a medium containing albumin).

Ovary stimulation

The indication for IVF in groups A and B was because of male factors (n = 17, n = 18), tubal factors (n = 9, n = 7) and polycystic ovary syndrome (n = 4, n = 5) respectively. Ovary stimulation was carried out for all women by a desensitizing protocol using GnRH-agonist (Suprefact D-65926, Hoechst AG, Germany). To suppress pituitary function, women were treated with Suprefact 0.5 mg/day given subcutaneously

for 14 days from the mid-luteal phase of the preceding cycle (day 20–22). As all women were ovulatory, they experienced menses with Suprefact treatment. After pituitary down regulation was confirmed by the absence of any ovarian follicle of > 10 mm in size, women underwent gonadotropin stimulation.

Induction of ovulation with human menopausal gonadotropin (HMG) (Menopur, Ferring, Germany) was started given intramuscularly in a dosage of 3 ampoules per day (215 UI of HMG) from day 2 for 7–12 days depending on the woman's response. The women were followed by vaginal sonography and monitoring of serum estradiol (E2) level. When serum E2 level was > 2000 pmol/L and at least 2 follicles were > 17 mm in diameter, 10 000 units of human chorionic gonadotropin (Organon, Holland) were administered intramuscularly. Transvaginal oocyte retrieval was performed 34–36 hours after hCG administration under transvaginal ultrasound guidance. The retrieved oocytes were then fertilized (in G-fert + albumin 10% for 36–40 hours) according to sperm quality either by conventional IVF (n = 10) or intracytoplasmic sperm injection (n = 20) in each group.

Patient selection and embryo transfer

Sixty (60) women were recruited on the day of embryo transfer and signed an informed consent and be included. The inclusion criteria were: age 35 years or younger, having at least 3 embryos suitable for transfer, and having no previous IVF embryo transfer cycle. The women were randomly divided into 2 groups. In group A (30 women), embryos were transferred to a medium supplemented with 0.5 mL/mL of hyaluronic acid (EmbryoGlue®, Vitrolife, Sweden) for 10 minutes before intrauterine transfer took place. In group B, that served as a control group (30 women), embryos were transferred as routinely done, to a medium containing albumin 20% (Bayer Corporation, United States) for 10 minutes. All embryo transfers were performed using a Labotect catheter (Labotect GmbH, Germany) on day 3 after oocyte retrieval.

The luteal phase was supported by progesterone in oil (Progestan, Nowr pharme-Neth, Tehran, Islamic Republic of Iran), 100 mg daily administered intramuscularly.

Patients were tested for serum β-hCG assay 14 days after embryo transfer. If the pregnancy test was positive, women were followed with serial ultrasounds to determine fetal viability. Clinical pregnancy was defined as the presence of a gestational sac on transvaginal ultrasound. Luteal phase support was continued until 10 weeks gestation.

Statistical analysis

Clinical results of the embryo transfer cycles were compared between the 2 groups. Data are presented as mean and standard deviation (SD). The results were analysed using the chi-squared test, Fisher exact test and ANOVA. P-value < 0.05 was considered significant.

Results

Table 1 shows some characteristics of the 2 study groups. Mean age (SD) was 27.5 (4.26) years for group A and was 28.60 (3.68) years for group B. Mean duration of infertility was 7.24 (3.68) years for group A and 6.93 (3.60) years for group B. Mean embryo transfer was 2.68 (0.66) for group A and 2.70 (0.79) for group B (Table 1).

In group A, 85 embryos were transferred resulting in 11 pregnancies and in group B, 98 embryos were transferred resulting in 7 pregnancies. The implantation rate was

Table 1 **Mean and standard deviation (SD) of some variables in the 2 groups**

Variable	Mean	SD	*P*-value
Age (years)			
Group A	27.50	4.26	0.786
Group B	28.60	3.68	
Duration of infertility (years)			
Group A	7.24	3.60	0.753
Group B	6.93	3.66	
Embryo number			
Group A	2.68	0.66	0.957
Group B	2.70	0.79	

Group A: medium supplemented with hyaluronic acid; Group B: medium supplemented with albumin.

36.7% in group A and 23.3% in group B, however this difference was not statistically significant ($P = 0.26$) (Table 2). All pregnancies were singleton. The abortion rate was higher in group B (28.6% versus 18.2%), but this was not statistically significant ($P \approx 1$) (Table 2).

Discussion

This study shows that hyaluronic acid can successfully replace albumin as a human embryo transfer medium resulting in comparable high pregnancy and implantation rates.

A study in 2000 showed that the embryos, which were developed in media supplemented with hyaluronic acid, had equivalent rates of blastocyst development and equivalent cell numbers compared to embryos cultured with albumin. In addition, the implantation and pregnancy rate were similar in the 2 groups [*8*].

Our results are similar to another study conducted in 2003 which suggested that the clinical pregnancy, implantation and ongoing pregnancy rates were higher with hyaluronic acid transfer medium compared with albumin transfer medium [*1*]. In another study a significant increase in both implantation and fetal development rates were reported when hyaluronic acid was the only macromolecule in the transfer medium of mouse embryos in comparison to transfer media that were combined with bovine serum albumin and hyaluronic acid [*14*].

The use of hyaluronic acid in the transfer medium may offer several advantages in the implantation process, such as a significant increase in both implantation and fetal development rates. Concern has been expressed about the immediate or late expulsion of embryos after their transfer to the uterine cavity [*15*]. However, our experience, as well as that of other researchers [*1,12,14*], suggests that the implantation and pregnancy rates were higher in the hyaluronic acid treatment group. We conclude, therefore, that hyaluronic acid medium can successfully replace albumin medium.

Table 2 **Outcome of pregnancy in the 2 groups**

Group	Implantation rate		Abortion rate		Ongoing pregnancy	
	No.	%	No.	%	No.	%
A	11/30	36.7	2/11	18.2	9/11	81.1
B	7/30	23.3	2/7	28.6	5/7	71.4
P-value	0.26		~1		~1	

Group A: medium supplemented with hyaluronic acid; Group B: medium supplemented with albumin.

References

1. Sbracia M et al. Hyaluronic acid substantially increases the retention of motility in cryopreserved/thawed human spermatozoa. *Human reproduction*, 1997, 12:1949–54.
2. Leese HJ. The formation and function of oviduct fluid. *Journal of reproduction and fertility*, 1988, 82:843–56.
3. Kemman E. Creutzfeldt–Jakob disease (CJD) and assisted reproductive technology (ART). Quantification of risks as part of informed consent. *Human reproduction*, 1998, 13:1777.
4. Van Os HC et al. The influence of contamination of culture medium with hepatitis B virus on the outcome of *in vitro* fertilization pregnancies. *American journal of obstetrics and gynecology*, 1991, 165:152–9.
5. Biggers JD, Summers MS, Mcginnis LK. Polyvinyl alcohol and amino acids as substitutes for bovine serum albumin in culture media for mouse preimplantation embryos. *Human reproduction update*, 1997, 3:125–35.
6. Furnus CC, de Matos DG, Martinez AG. Effect of hyaluronic acid on development of *in vitro* produced bovine embryos. *Theriogenology*, 1998, 49:1489–99.
7. Gardner DK, Rodriegez-Martinez ZH, Lane M. Fetal development after transfer is increased by replacing protein with the glycosaminoglycan hyaluronan for mouse embryo culture and transfer. *Human reproduction*, 1999, 14:2575–80.
8. Gardner DK, Lane M. Recombinant human serum, albumin and hyaluronan can replace blood-derived albumin in embryo culture media. *Fertility and sterility*, 2000, 74(suppl.):S31–2.
9. Gardner DK, Lane M. Culture of viable human blastocysts in defined sequential serum-free media. *Human reproduction*, 1998, 13(suppl.):148–60.
10. Salustri A et al. Hyaluronan and proteoglycans in ovarian follicles. *Human reproduction update*, 1999, 5:293–301.
11. Lane M et al. Cryo-survival and development of bovine blastocysts are enhanced by culture with recombinant albumin and hyaluronan. *Molecular reproduction and development*, 2003, 64:70–80.
12. Stojkovic M et al. Effects of high concentrations of hyaluronan in culture medium on development and survival rates of fresh and frozen–thawed bovine embryos produced *in vitro*. *Reproduction*, 2003, 124:141–53.
13. Simon A et al. Hyaluronic acid can successfully replace albumin as the sole macromolecule in human embryo transfer medium. *Fertility and sterility*, 2003, 79:1434–8.
14. Gardner DR, Rodriegez-Martinez H, Lane M. Fetal development after transfer is increased by replacing protein with the glycosaminoglycan hyaluronan for mouse embryo culture and transfer. *Human reproduction*, 1999, 14:2575–80.
15. Mansour RT, Aboulghar MA. Optimizing the embryo transfer technique. *Human reproduction*, 2002, 17:1149–53.