ORIGINAL ARTICLE

Effect of intrauterine injection of human chorionic gonadotropin before embryo transfer on pregnancy rate: A prospective randomized study

Fatemeh Mostajeran, Farzaneh Godazandeh, Sayed Mehdi Ahmadi[1], Minoo Movahedi, Seyed Abolfazl Jabalamelian[2]
Department of Obstetric and Gynecology, Faculty of Medicine, Isfahan University of Medical Sciences, [1]Isfahan Infertility Center, [2]Department of Counseling, School of Psychology, University of Isfahan, Isfahan, Iran

Background: Human chorionic gonadotropin (hCG) as the most important factor to controlled implantation is one of the early embryonic signals in primates that is secreted by the embryo before its implantation. This study was designed to assess the effects of intrauterine injection of hCG before the embryo transfer in an *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycle on pregnancy rate in infertile patients. **Materials and Methods:** This randomized study was done on 100 infertile patients in two groups: intervention group received injection of 700 IU of intrauterine hCG 10 min before embryo transfer and control group did not receive hCG. The pregnancy rate was tested 2 weeks after embryo transfer, and if the pregnancy test was positive, a transvaginal ultrasound was performed 3 weeks later to search for signs of pregnancy, such as the presence of a gestational sac, embryo, and fetal heart rate, and confirmed as successful pregnancy. **Results:** Pregnancy test was positive in 13 (28.6%) of 46 patients in hCG group and in control group was positive in 6 (12.5%) of 48 patients. The pregnancy rate between hCG group and control group was not significantly different (P = 0.54). The pregnancy rate in hCG group with IVF fertilization was 20.8% and in their controls was 7.4% (P = 0.51). The pregnancy rate in hCG group with ICSI fertilization was 36.4% and in their controls was 19% (P = 0.16). **Conclusion:** The intrauterine injection of 700 IU of hCG before embryo transfer improved pregnancy rate compared to control group but was not significantly different.

Key words: Embryo transfers, human chorionic gonadotropin, infertility, *in vitro* fertilization, intracytoplasmic sperm injection

How to cite this article: Mostajeran F, Godazandeh F, Ahmadi SM, Movahedi M, Jabalamelian A. Effect of intrauterine injection of human chorionic gonadotropin before embryo transfer on pregnancy rate: A prospective randomized study. J Res Med Sci 2017;22:6.

INTRODUCTION

It is estimated that one out of seven couples worldwide and 15% of the couples in developed countries are suffering from infertility.[1,2] *In vitro* fertilization (IVF) as the main treatments in infertile couples is a highly complex technique that involves the use of standardized protocols for a controlled ovarian stimulation, oocyte retrieval under ultrasound guidance, fertilization of gametes in the laboratory, embryo culturing, and embryo transfer.[3] The first step in IVF/intracytoplasmic sperm injection (ICSI) treatment is controlled ovarian stimulation with gonadotropins during each cycle.[4,5]

Access this article online

Quick Response Code:

Website: www.jmsjournal.net

DOI: 10.4103/1735-1995.199096

The success rate for IVF reported range between 40.1% (in women <35 years) and 20.6% (in women in the 41–42 years).[6] Successful implantation after IVF and ICSI cycles depends on various factors and requires precise synchronization between the embryo and the uterine environment.[7-9] It is estimated that failure of implantation is the cause of approximately 50%–75% of lost pregnancies.[10]

Human chorionic gonadotropin (hCG) is one of the early embryonic signals in primates that is secreted by the embryo before its implantation and is the most important factor to controlled implantation.[11,12] HCG is a heterodimeric placental glycoprotein hormone that is required to maintain pregnancy and is initially produced by the blastocyst 6–8 days after fertilization.[13,14]

Address for correspondence: Dr. Farzaneh Godazandeh, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: fgodazandeh@yahoo.com
Received: 10-02-2016; **Revised:** 28-08-2016; **Accepted:** 26-10-2016

Correlation between implantation rate and the beta-hCG concentration in IVF cycles is assessed in some studies. One study reported a positive correlation between the beta-hCG concentration and the implantation rate; also, other studies showed a positive correlation and significant increase in the clinical pregnancy rate after intrauterine injection of hCG before the embryo transfers.[15-17] Different mechanisms have been described for effect of hCG in regulation of implantation. One of these mechanisms is that hCG is a potent attractor of inflammatory cells, such as neutrophils, monocytes, and lymphocytes. One of these mechanisms is that hCG directly regulates endothelial cell responsiveness to interleukin 1 (IL-1) and amplifies the cytokine-mediated effect on cell proliferation, migration, and release of angiogenic factors. In addition, it is reported that hCG displayed a potent angiogenic effect through receptor activation of transforming growth factor beta in endothelial cells, which is a key role in placental development.

More clinical trials are suggested to evaluate the effect of intrauterine injection of hCG before the embryo transfers in IVF and ICSI cycles in infertile women, and also in most of the previous studies, hCG is used in the dose of 500 IU or lower and there is no information about the effects of injection of 700 IU of intrauterine hCG; hence, in comparison with previous studies, the present study was designed to assess the effects of higher dose of intrauterine injection of hCG before the embryo transfer in an IVF or ICSI cycles on pregnancy rate in comparison with control.

MATERIALS AND METHODS

This randomized, parallel-group, single blind study was conducted from September 2013 to April 2014, on 100 infertile women under the age of 40 years, at the Fertility and Infertility Center of Isfahan in Iran. Women of 20–40 years old with body mass index 18–30 kg/m^2 were eligible if they were infertile due to male factor, had a regular menstrual cycle of 24–35 days, and presumed to be ovulatory. Patients with the presence of polycystic ovary syndrome, with uterine pathologies, endometriosis, or the presence of hydrosalpinges and any endocrine disease or chronic systemic illness were excluded from the study. Furthermore, the exclusion criteria were azoospermia and the history of successful IVF or ICSI. The Ethics Committee of Isfahan University of Medical Sciences approved this study (project number, 392387). The patients were counseled and informed about the study protocol and if they were willing, after given written informed consent were entered into the study.

Eligible patients were randomly divided into two fifty-member groups by Random Allocation software (Saghaei, 2004). Group A as the intervention group received injection of 700 IU of intrauterine hCG (Chorionic Gonadotropin Human, Darou Pakhsh Company, Iran) 10 min before embryo transfer. Group B as the control group did not receive HCG before embryo transfer. In intervention group to embryo transfer, the patient was put in the lithotomy position, and the cervix was visualized using Cusco's speculum. The cervical mucus was wiped out using a sterile piece of gauze, and then the mucous was partially removed by gentle suction with a 1-ml syringe. The HCG for intrauterine injection was prepared by adding 5 ml of tissue culture media to one vial that containing 700 IU of hCG being injected intrauterine after the catheter had passed the internal cervical ovarian stimulation. The embryos were loaded into another embryo transfer catheter and were transferred into the uterine cavity, around 5–10 min after the hCG intrauterine injection.[9] Then, 5 min after the embryo transfer, the vaginal speculum was left in place and was removed. In control group, embryo transfer was done like intervention group without any injection of hCG. All embryos were embryos frozen at the blastocyst stage and the embryo transfer in both groups was done by the attending gynecologist who was blinded to the study. The number of blastocysts transferred on any occasion was not than three which was determined by availability of embryos, patient age, and patient's previous clinical history.

Collected data included age, duration of infertility, history of implantation failure, fertilization technique (IVF or ICSI), and the pregnancy rate. The pregnancy test was done in all patients 2 weeks after embryo transfer, and if the pregnancy test was positive, a transvaginal ultrasound was performed 3 weeks later to search for signs of pregnancy, such as the presence of a gestational sac, embryo, and fetal heart rate, which confirmed as successful pregnancy.

The sample size was calculated using the comparison of proportion formula with two-sided log-rank test, $\alpha = 0.05$, and 84% power. All statistical analyses were done using SPSS software (SPSS Inc, Chicago, IL, USA) for Windows, version 20. Descriptive data are reported as mean ± standard deviation or number (percent) as appropriate. Independent sample *t*-test was used to compare women and their husbands' age, body mass index, duration of infertility, mean of number of mature oocytes, oocytes retrieved, fertilized oocytes, and embryos transferred. And also, Chi-square test was used to compare history of implantation failure, fertilization technique, and pregnancy rate between intervention and control groups. Statistical significance was accepted at $P < 0.05$.

RESULTS

Figure 1 compares the flowchart of the study. Of 112 screened patients for eligibility, seven patients were not eligible and five patients refused informed consent and did not enter into the study. One-hundred patients were

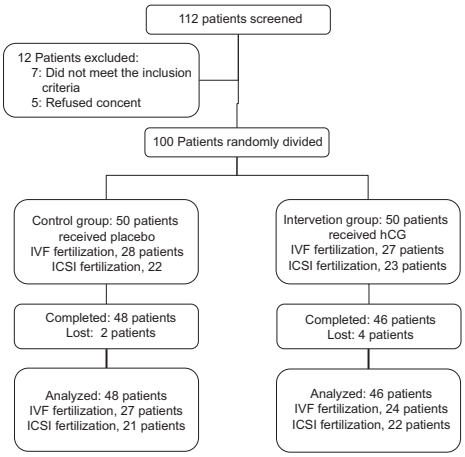

Figure 1: Flowchart of the study

eligible and randomly assigned into two intervention and control groups. Six patients did not refer to sonography in follow-up period, calls not being answered, and lost to follow-up. Finally, 94 patients completed the study and analyzed (46 patients in intervention group and 48 in control group).

The mean age of the studied patients was 31.3 ± 5.2 years, with mean infertility duration of 36.9 ± 22.7 months. Table 1 shows baseline characteristics of studied patients by groups. No significant differences were noted between intervention group and control group for mean of women and husbands' age, body mass index, duration of infertility, history of implantation failure, and fertilization technique (IVF or ICSI), and also, the number of mature oocytes, oocytes retrieved, fertilized oocytes, and embryos transferred between intervention group and control group were not statistically significant ($P \geq 0.5$).

Table 2 shows the pregnancy rate based on sonography after injection of 700 UI of intrauterine hCG in comparison with control group. In hCG group of 46 studied patients, pregnancy test was positive in 24 patients (pregnancy rate 52.1%), and in control group of 48 patients, pregnancy test was positive in 27 patients (pregnancy rate 56.2%). The pregnancy rate between hCG group and control group was not significantly different ($P = 0.54$).

Table 3 shows the pregnancy rate based on sonography after injection of 700 UI of intrauterine hCG in comparison with control group in regard to infertility treatment methods. IVF fertilization was done in 24 (52.1%) of 46 patients in hCG group, in these patients, pregnancy rate was 20.8%, and in control group, IVF fertilization was done in 27 (56.2%) of 48 patients with pregnancy rate of 7.4%. There was no significant difference between studied group for pregnancy rate under IVF fertilization ($P = 0.51$). ICSI fertilization was done in 47.9% of patients in hCG group with pregnancy rate of 36.4%, and in control group, ICSI fertilization was done in 43.8% of patients with pregnancy rate of 19%. There was no

Table 1: Baseline characteristics in studies patients by groups

	hCG group	Control group	*P*
Age (years)			
IVF fertilization	31.9±6.4	32.5±4.5	0.7*
ICSI fertilization	31.3±5.1	29.2±4.5	0.16*
Husband age			
IVF fertilization	36.2±8.9	37.5±3.9	0.51*
ICSI fertilization	38.3±6.2	38.1±5.1	0.88*
BMI			
IVF fertilization	24.2±4	25.1±3.1	0.4*
ICSI fertilization	23.5±5.3	23.1±5.6	0.8*
Duration of infertility (month)			
IVF fertilization	49.7±31.4	38.2±15.9	0.1*
ICSI fertilization	30.3±23.3	28.3±8.3	0.71*
History of implantation failure			
IVF fertilization	10 (43.5)	9 (33.3)	0.46†
ICSI fertilization	5 (21.7)	5 (23.8)	0.87†
Number of mature oocytes			
IVF fertilization	12.1±5.3	10.9±5.4	0.61*
ICSI fertilization	10.7±4.8	9.8±5.1	0.73*
Number of oocytes retrieved			
IVF fertilization	7.7±4.1	8.2±3.8	0.65*
ICSI fertilization	8.8±3.3	6.7±4.4	0.41*
Number of embryos transferred			
IVF fertilization	1.4±0.73	1.7±0.71	0.19*
ICSI fertilization	1±0.32	1±0.55	0.2*
Number of fertilized oocytes			
IVF fertilization	3.1±3	2.4±3.5	0.49*
ICSI fertilization	2±2.1	1.7±2.8	0.11*

Data expressed as mean±SD or *n* (%). *P* values calculated by *Independent sample *t*-test, †Chi-square test. hCG = Human chorionic gonadotropin; SD = Standard deviation; IVF = *In vitro* fertilization; ICSI = Intracytoplasmic sperm injection; BMI = Body mass index

Table 2: Comparison of pregnancy rate in studies patients by groups

	hCG group	Control group	*P*
Positive	24 (52.1)	27 (56.2)	0.54
Negative	22 (47.9)	21 (43.8)	

Data expressed as *n* (%). *P* value calculated by Chi-square test. hCG = Human chorionic gonadotropin

Table 3: Comparison of pregnancy rate base on sonography between studies groups in regard to infertility treatment methods

	hCG group	Control group	*P*
IVF fertilization			
Positive	5 (20.8)	2 (7.4)	0.51
Negative	19 (79.2)	25 (92.6)	
ICSI fertilization			
Positive	8 (36.4)	4 (19)	0.16
Negative	14 (63.6)	17 (81)	

Data expressed as *n* (%). *P* values calculated by Chi-square test. hCG = Human chorionic gonadotropin; IVF = *In vitro* fertilization; ICSI = Intracytoplasmic sperm injection

significant difference between studied group for pregnancy rate under ICSI fertilization treatment method ($P = 0.16$).

DISCUSSION

It is shown that there is a key role for hCG in regulating the inflammatory response and angiogenesis during embryo implantation, and an altered damaged endometrial receptivity by the IVF treatments can be overcome by injecting hCG before the embryo transfers.[18-20] In this randomized clinical trial, we assessed the effects of intrauterine hCG injection before transferring the embryos on the outcome of the IVF or ICSI cycles and our results showed that the pregnancy rate, based on sonography, after injection of 700 UI of intrauterine hCG before embryo transfer was higher than in control group (28.6% vs. 12.5%, respectively), but the difference between groups was not significantly different. In addition, no significant association was found between pregnancy rate and infertility treatment technique. The rate of pregnancy in our study is low which can be explained by the presence of patients with history of implantation failure whereas nearly 30% of cases and controls reported history of implantation failure.

Embryonic implantation would be successful if a communication link occurred between the embryo and its near environment within the implantation site. The pharmacokinetics of hCG could be known as the underlying mechanism of effect of intrauterine injection of hCG to improvement implantation and pregnancy outcome. Previously, an increase in the endometrial cell proliferation and migration is shown in response to administration of hCG. Embryo implantation enhances by increases of IL-8 and it is shown that IL-1R2, which in turn increases the secretion of IL-8, significantly downregulates after administration of hCG, and either IL-1R1 would be increased.[21] Positive correlation between the serum level of hCG with the level of trophoblast tolerance as well as the number of uterine natural killer cells is shown previously.[13] And also, hCG as a trophoblast marker which is secreted by blastocyst before and after implantation is shown in some previous studies.[22-24] hCG is a potent attractor of inflammatory cells, such as neutrophils, monocytes, and lymphocytes. It directly regulates endothelial cell responsiveness to IL-1 by hCG and amplifies the cytokine-mediated effect on cell proliferation, migration, and release of angiogenic factors, and angiogenic effects of hCG through receptor activation of transforming growth factor beta in endothelial cells are the possible mechanisms of hCG to improve implantation.

There are few clinical studies that previously assessed the effects of intrauterine hCG injection before transferring the embryos on the outcome of the IVF or ICSI cycles. In the first study by Mansour *et al*.,[15] intrauterine injection of 100, 200, and 500 IU of hCG before embryo transfer was compared to control group. In this study, 167 patients in two groups received intrauterine injection of 100 or 200 IU of hCG before embryo transfer and pregnancy rate in these groups was assessed compared to control group. They could not found any statistically significant difference between the intervention groups and control group (pregnancy rate was 54% in the 100 hCG group, 57% in the 200 IU hCG group, and 60% in the control group). The pregnancy rate in 500 IU of hCG group (75%) has been significantly higher as compared with the control group (60%). Mansour *et al*. showed that higher dose of hCG has been better than lower dose. In the present study, higher dose of hCG in comparison with Mansour *et al*.'s study has been assessed, but we did not find a significant difference between intrauterine injection of 700 IU of hCG in comparison with control group, which was in contrast to the findings in Mansour *et al*.'s study.[16] The pregnancy rate in hCG and control groups in our study was lower than all studied groups in Mansour *et al*.'s study.[16] The differences between results of Mansour *et al*.'s study and our study could be explained by difference in inclusion criteria for studied patients, whereas in the present study, history of recurrent miscarriage and implantation failure was not known as inclusion or exclusion criteria and our study included patients with or without a history of recurrent miscarriage and implantation failure. On the other hand, in Mansour *et al*.'s study, patients with or without a history of recurrent miscarriage and implantation failure have been excluded and only the first-time IVF patients have been registered.[16]

In Zarei *et al*.'s study,[25] pregnancy rate in patients who received 250 μg intrauterine rhCG before embryo transfer was 32.1% which was significantly higher than 18.4% in those who received placebo. In comparison with our study, pregnancy rate after injection of intrauterine hCG before embryo transfer was 28.6% and in control group was 12.55%. Another prospective randomized study by Santibañez *et al*. was done on 210 infertile patients with a history of recurrent miscarriage and implantation failure. One-hundred and one patients received an intrauterine injection of 500 IU of hCG before the embryo transfer, and pregnancy rate in this intervention group was compared with pregnancy rate in 109 patients who did not receive hCG. The pregnancy rate in hCG group was 50.4% and was significantly higher than in control group (33%).[16] In contrast to Zarei *et al*. and Santibañez *et al*. studies, our finding did not report significant difference between hCG and controls. In our study, pregnancy rate was lower than in these two studies.[16,25] The differences between these results and our study may be explained by differences in sample size and time of injection. In addition, use of thawed embryo transfers in Santibañez *et al*. is another possible cause of difference between findings,[16] whereas the benefits

of hCG injection were also shown in this group of patients, and also Santibañez *et al*. reported that pregnancy rate was not their primary outcome and the statistical analyses were not designed for this approach.

However, the pregnancy rate after injection of 700 UI of intrauterine hCG before embryo transfer in our study was not significantly different with control group but in hCG group was more than double of these rate in the control group (28.6% vs. 12.5%, respectively), and these results with findings of previous studies[16,17] showed that intrauterine injection of hCG before the embryo transfer as a simple procedure can be useful to improve pregnancy rates in infertile women underwent IVF or ICSI treatment. On the other hand, this method has some advantages: intrauterine injection of hCG is not expensive and is a cost-effective method in infertile women. Other advantage is that this method does not consume additional time for the embryologist and clinical staff and does not require complex training. In addition, this intervention could be replicated by all units and could be considered to be without major risk. Hence, advantages of this method beside of positive effects of hCG to enhance pregnancy rate could make it as good intervention to treatment infertile women.

CONCLUSION

Our results showed that the intrauterine injection of 700 IU of hCG before embryo transfer improved pregnancy rate compared to control group but was not significantly different. The effects of intrauterine injection of hCG before embryo on pregnancy rate is studied in a few trails with different dosage and different result, so more clinical trials are needed to elucidate these results for this intervention.

Acknowledgments

This study was financially supported by the Isfahan University of Medical Sciences, Isfahan, Iran. We gratefully acknowledge the dedicated efforts of the investigators, coordinators, volunteer patients who participated in this study.

Financial support and sponsorship

This study was supported by the Isfahan University of Medical Sciences, Isfahan, Iran.

Conflicts of interest

There are no conflicts of interest.

AUTHORS' CONTRIBUTION

FM contributed in the design of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. FG contributed in the design of the work, conducting the study analysis of data for the work, drafting and revising, approval of the final version of the manuscript, and agreed for all aspects of the work. SMA contributed in the conception of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MM contributed in the conception of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AJ contributed in conducting the study, analysis of data for the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

REFERENCES

1. Kably Ambe A, López Ortiz CS, Serviere Zaragoza C, Velázquez Cornejo G, Pérez Peña E, Santos Haliscack R, *et al.* Mexican national consensus on assisted reproduction treatment. Ginecol Obstet Mex 2012;80:581-624.
2. Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. N Engl J Med 2001;345:1400-8.
3. Bourdiec A, Shao R, Rao CV, Akoum A. Human chorionic gonadotropin triggers angiogenesis via the modulation of endometrial stromal cell responsiveness to interleukin 1: A new possible mechanism underlying embryo implantation. Biol Reprod 2012;87:66.
4. Berndt S, Blacher S, Munaut C, Detilleux J, Perrier d'Hauterive S, Huhtaniemi I, *et al.* Hyperglycosylated human chorionic gonadotropin stimulates angiogenesis through TGF-ß receptor activation. FASEB J 2013;27:1309-21.
5. Licht P, Fluhr H, Neuwinger J, Wallwiener D, Wildt L. Is human chorionic gonadotropin directly involved in the regulation of human implantation? Mol Cell Endocrinol 2007;269:85-92.
6. Tsampalas M, Gridelet V, Berndt S, Foidart JM, Geenen V, Perrier d'Hauterive S. Human chorionic gonadotropin: A hormone with immunological and angiogenic properties. J Reprod Immunol 2010;85:93-8.
7. Zenclussen AC, Gerlof K, Zenclussen ML, Ritschel S, Zambon Bertoja A, Fest S, *et al.* Regulatory T cells induce a privileged tolerant microenvironment at the fetal-maternal interface. Eur J Immunol 2006;36:82-94.
8. Licht P, Lösch A, Dittrich R, Neuwinger J, Siebzehnrübl E, Wildt L. Novel insights into human endometrial paracrinology and embryo-maternal communication by intrauterine microdialysis. Hum Reprod Update 1998;4:532-8.
9. Mansour R, Fahmy I, Tawab NA, Kamal A, El-Demery Y, Aboulghar M, *et al.* Electrical activation of oocytes after intracytoplasmic sperm injection: A controlled randomized study. Fertil Steril 2009;91:133-9.
10. Cole LA. hCG and hyperglycosylated hCG in the establishment and evolution of hemochorial placentation. J Reprod Immunol 2009;82:112-8.
11. Kane N, Kelly R, Saunders PT, Critchley HO. Proliferation of uterine natural killer cells is induced by human chorionic gonadotropin and mediated via the mannose receptor. Endocrinology 2009;150:2882-8.
12. Wan H, Versnel MA, Cheung WY, Leenen PJ, Khan NA, Benner R, *et al.* Chorionic gonadotropin can enhance innate immunity by stimulating macrophage function. J Leukoc Biol 2007;82:926-33.
13. Sugihara K, Kabir-Salmani M, Byrne J, Wolf DP, Lessey B, Iwashita M, *et al.* Induction of trophinin in human endometrial surface epithelia by CGbeta and IL-1 beta. FEBS Lett 2008;582:197-202.

14. Xiao-Yan C, Jie L, Dang J, Tao L, Xin-Ru L, Guang-Lun Z. A highly sensitive electrochemiluminescence immunoassay for detecting human embryonic human chorionic gonadotropin in spent embryo culture media during IVF-ET cycle. J Assist Reprod Genet 2013;30:377-82.
15. Mansour R, Tawab N, Kamal O, El-Faissal Y, Serour A, Aboulghar M, *et al.* Intrauterine injection of human chorionic gonadotropin before embryo transfer significantly improves the implantation and pregnancy rates in *in vitro* fertilization/intracytoplasmic sperm injection: A prospective randomized study. Fertil Steril 2011;96:1370-4.e1.
16. Santibañez A, García J, Pashkova O, Colín O, Castellanos G, Sánchez AP, *et al.* Effect of intrauterine injection of human chorionic gonadotropin before embryo transfer on clinical pregnancy rates from *in vitro* fertilisation cycles: A prospective study. Reprod Biol Endocrinol 2014;12:9.
17. Környei JL, Lei ZM, Rao CV. Human myometrial smooth muscle cells are novel targets of direct regulation by human chorionic gonadotropin. Biol Reprod 1993;49:1149-57.
18. Lee TK, Kim DI, Song YL, Lee YC, Kim HM, Kim CH. Differential inhibition of *Scutellaria barbata* D. Don (*Lamiaceae*) on HCG-promoted proliferation of cultured uterine leiomyomal and myometrial smooth muscle cells. Immunopharmacol Immunotoxicol 2004;26:329-42.
19. Schumacher A, Brachwitz N, Sohr S, Engeland K, Langwisch S, Dolaptchieva M, *et al.* Human chorionic gonadotropin attracts regulatory T cells into the fetal-maternal interface during early human pregnancy. J Immunol 2009;182:5488-97.
20. Banerjee P, Fazleabas AT. Endometrial responses to embryonic signals in the primate. Int J Dev Biol 2010;54:295-302.
21. Bourdiec A, Bédard D, Rao CV, Akoum A. Human chorionic gonadotropin regulates endothelial cell responsiveness to interleukin 1 and amplifies the cytokine-mediated effect on cell proliferation, migration and the release of angiogenic factors. Am J Reprod Immunol 2013;70:127-38.
22. Lopata A, Hay DL. The potential of early human embryos to form blastocysts, hatch from their zona and secrete HCG in culture. Hum Reprod 1989;4 8 Suppl: 87-94.
23. Hoshina M, Boothby M, Hussa R, Pattillo R, Camel HM, Boime I. Linkage of human chorionic gonadotrophin and placental lactogen biosynthesis to trophoblast differentiation and tumorigenesis. Placenta 1985;6:163-72.
24. Bonduelle ML, Dodd R, Liebaers I, Van Steirteghem A, Williamson R, Akhurst R. Chorionic gonadotrophin-beta mRNA, a trophoblast marker, is expressed in human 8-cell embryos derived from tripronucleate zygotes. Hum Reprod 1988;3:909-14.
25. Zarei A, Parsanezhad ME, Younesi M, Alborzi S, Zolghadri J, Samsami A, *et al.* Intrauterine administration of recombinant human chorionic gonadotropin before embryo transfer on outcome of *in vitro* fertilization/intracytoplasmic sperm injection: A randomized clinical trial. Iran J Reprod Med 2014;12:1-6.