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ORIGINAL ARTICLE *Infertility*

Intrauterine administration of hCG immediately after oocyte retrieval and the outcome of ICSI: a randomized controlled trial

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STUDY QUESTION: Does the intrauterine administration of hCG immediately after oocyte retrieval in antagonist cycles with ICSI and fresh embryo transfer (ET) influence the implantation rate or chemical and clinical pregnancy rates?

SUMMARY ANSWER: The intrauterine administration of hCG after oocyte retrieval increases the implantation rate and chemical and clinical pregnancy rates.

WHAT IS KNOWN ALREADY: Over half of IVF/ICSI cycles fail due to implantation failure. Intrauterine administration of hCG, a few minutes before ET, increased the implantation and pregnancy rates in most but not in all studies. The effect of intrauterine administration of hCG, after oocyte retrieval, has not yet been studied.

STUDY DESIGN, SIZE, DURATION: The study was a parallel, triple-blind randomized clinical trial (RCT) performed from September 2015 to February 2016, in a university hospital. We recruited women undergoing antagonist ovarian stimulation, ICSI and ET. For an effect size of 0.2, power of 80% at a significance level of 0.05, we needed 150 participants. Accounting for a 7% dropout rate, a total of 160 women was considered appropriate. A computer-generated randomization list with a block size of 4, with 1:1 allocation was used. The treatment allocation was placed in a sealed, opaque, envelope and picked up consecutively. Immediately after oocyte retrieval, patients in the intervention and control groups were treated with intrauterine injection of hCG and saline, respectively. Participants underwent ET on Day 3. A beta-hCG test was done at 2 weeks. If positive, three transvaginal-ultrasonographies (TVSs) were done at 3, 4 and 10 weeks after ET. The participants were called up thereafter and questioned about the continuity of their pregnancy.

PARTICIPANTS/MATERIALS, SETTING, METHOD: Of 1990 women attending the infertility clinic of our university hospital, 508 were IVF/ICSI candidates during the study period, and 245 of the patients on an antagonist cycle met the criteria to be invited into our trial. Inclusion criteria were normal ovarian reserve, age ≤41, undergoing ICSI, and fresh ET and normal TSH and prolactin. Uncontrolled chronic disease, severe hydrosalpinx, severe endometriosis, morphologic embryo deficiencies, non-obstructive azospermia and high risk of severe ovarian hyperstimulation syndrome were criteria for exclusion. After taking an informed consent, a total of 158 participants were recruited, of which 80 were randomly allocated to receive intrauterine 500 IU hCG in up to 0.5 ml normal saline and 78 to receive intrauterine 0.5 ml normal saline immediately after oocyte retrieval, during general anaesthesia. ICSI was performed conventionally. The 4–8 cell embryos were transferred on the third day after oocyte retrieval. Implantation rate, chemical and clinical pregnancy rates were analysed and compared between the two groups.

MAIN RESULTS AND THE ROLE OF CHANCE: Patients' demographic and baseline characteristics were comparable. The clinical results showed statistically significant differences between the two groups regarding the biochemical pregnancy rate (59.2 versus 31.3%; $P = 0.001$; odds ratio (OR) = 1.88; 95% CI, 1.26–2.82; risk difference (RD) = 27.8; 95% CI, 11.2–42.3), implantation rate (37 versus 17%; $P = 0.012$; OR = 2.29; 95% CI, 1.02–5.14; RD = 20.2; 95% CI, 5.4–33.8), clinical pregnancy rate (50.7 versus 16.4%; $P < 0.001$; OR = 3.08;

†The authors consider that the first two authors should be regarded as joint first authors.

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95% CI, 1.71–5.55; RD = 34.3; 95% CI, 18.7–47.6) and ongoing pregnancy rate (40.1 versus 13.4%; $P = 0.001$; OR = 3.04; 95% CI, 1.55–5.93; RD = 27.4; 95% CI, 12.7–40.6). The abortion and ectopic pregnancy rates were not statistically different between the two groups.

LIMITATIONS, REASONS FOR CAUTION: The insertion of an intrauterine insemination catheter and the injection of a small amount of saline into the uterine cavity (without hCG) may also have some impact on implantation. This effect could be studied by comparing this intervention with another study group without any intrauterine injection.

There are no specific side effects mentioned in the literature for the intrauterine administration of hCG, neither were any observed in our study, but it is best to be cautious about probable side effects, because this type of intervention is relatively new and experimental, and deserves more studies before being entered into routine clinical practice.

WIDER IMPLICATIONS OF THE FINDINGS: Intrauterine administration of hCG immediately after oocyte pick up increases its effectiveness; however, further investigations are required before this procedure can be recommended for clinical practice.

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Introduction

Implantation failure is the cause of more than half of the pregnancy failures in ART cycles (Mansour *et al.*, 2011; Wirleitner *et al.*, 2015). Implantation is a complex process that is regulated by many autocrine and paracrine factors. hCG is one of the most important of these factors. Embryos secrete hCG before their implantation (Licht *et al.*, 2007; Cole, 2012; Wirleitner *et al.*, 2015), hCG transcription beginning from the two-cell stage. It is also produced from the endometrial epithelial cells, mainly during the luteal phase (Bourdiec *et al.*, 2013b). Using an intrauterine microdialysis system to release low concentrations of hCG to the endometrium in the luteal phase, Licht *et al.* (2007) showed that hCG acts before implantation, in an autocrine-juxtacrine manner until its appearance in the serum. Intrauterine hCG administration modulated paracrine factors of differentiation (e.g. prolactin, insulin-like growth factor binding protein-1 (IGFBP-1)) and of implantation (e.g. leukaemia inhibitory factor (LIF) and macrophage colony stimulating factor (M-CSF)). Vascular endothelial growth factor (VEGF) and matrix metalloproteinase 9 (MMP-9), important for tissue remodelling, were increased by hCG, suggesting an essential role for hCG in angiogenesis, vascularization and placentation of the endometrium (Licht *et al.*, 2001; Cole, 2012). HCG modulates endometrial cell and endothelial cell response to interleukin1, one of the earliest embryonic signals, and attracts T regulatory cells to trophoblasts; so in the endometrium, hCG promotes gene expression towards implantation, receptivity and tolerance (Cole, 2010, 2012; Bourdiec *et al.*, 2013b; Schumacher *et al.*, 2014).

Using laser capture microdissection, Palomino *et al.* (2013) demonstrated that the gene expression levels of endometrial complement C3 and Decay-Accelerating Factor are modulated by intrauterine administration of hCG. In addition, crosstalk exists between the intrauterine hCG signal and the ovarian steroid hormones. These hormones regulate the expression of endometrial complement proteins. Therefore, hCG secreted by the embryo may function as a modulator of embryo-maternal communication and maternal immune response during implantation (Palomino *et al.*, 2013; Bourdiec *et al.*, 2013a).

Culturing the embryos *in vitro* decreases hCG signalling to the endometrium during the early days of the embryo development and could contribute to the relatively low implantation rate in ART cycles (Wirleitner *et al.*, 2015; Strug *et al.*, 2016). Many studies have shown the effectiveness of intrauterine hCG injection before cleavage-stage embryo transfer (ET) (Mansour *et al.*, 2011; Santibañez *et al.*, 2014; Zarei *et al.*, 2014). However, others showed no beneficial effect before blastocyst transfer (Hong *et al.*, 2014; Wirleitner *et al.*, 2015). The reason could be that with the blastocyst transfer, there is not enough time for hCG to have any beneficial effect on the endometrium before implantation (Licht *et al.*, 2007; Strug *et al.*, 2016).

To overcome the limitations of the short duration of the intrauterine hCG effect before ET, we planned a study to administer intrauterine hCG earlier than those mentioned in previous studies. Also to bypass the stress and discomfort of another procedure for the infertile patient, we supposed that the best time to administer intrauterine hCG might be immediately after oocyte retrieval, during general anaesthesia. Therefore, we designed a study, in fresh antagonist cycles of IVF/ICSI, to assess the value of intrauterine administration of hCG after oocyte pick up, on the implantation and pregnancy rates. To date, there are not any published studies demonstrating the impact of intrauterine hCG administration immediately after oocyte retrieval on the outcome of the IVF/ICSI cycles.

Materials and Methods

The study proposal was approved by the ethics committee of our university centre. From September 2015 to February 2016, 1990 women attended the infertility clinic of our university hospital, and 508 of them were IVF/ICSI candidates. A total of 158 of 245 women on an antagonist cycle, who had the criteria to be included in the trial, accepted our invitation to participate in the study and signed an informed consent. Inclusion criteria were normal ovarian reserve (anti-Müllerian hormone ≥0.7 ng/ml), age ≤41 years, undergoing ICSI and fresh ET and normal level of thyroid stimulating hormone and prolactin. Exclusion criteria were uncontrolled chronic maternal

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diseases like endocrinopathies and autoimmune diseases, severe endometriosis, severe hydrosalpinx, non-obstructive azoospermia, high risk for severe ovarian hyperstimulation syndrome (development of >20 follicles >10 mm at ovarian stimulation or retrieval of >16 oocytes at the day of oocyte retrieval) (Broer *et al.*, 2013) and morphologic embryo deficiencies. All participants were on a flexible antagonist protocol (Weissman *et al.*, 2012) for controlled ovarian stimulation (COS). When at least three follicles ≥17 mm were seen by transvaginal ultrasonography (TVS), final oocyte maturation was triggered by an intramuscular injection of 10 000 IU hCG (Choriomon, IBSA, Switzerland). Oocyte retrieval was planned 36–40 h later.

Five thousand IU hCG (Choriomon, IBSA) were dissolved in 1 ml distilled water and stored in the refrigerator at 4°C. For each patient in the intervention group, 0.1 ml (500 IU hCG) and 0.4 ml normal saline were pulled into an insulin syringe, and for the patients in the control group, 0.5 ml normal saline. Immediately after oocyte retrieval, during general anaesthesia, a vaginal speculum was put in place. Any bleeding and clots were cleaned, and the cervix was visualized. The drug to be injected was handed to the physician by an operating room technician, who was the only person who was aware of the patient allocation and the content of the syringe. The physician injected the solution through an intrauterine insemination catheter, into the uterine cavity, 1–2 cm above the internal ostium of the cervix and withdrew the catheter 2 s later. The patient and the physician were blinded for the type of intervention. The ICSI and ET were performed conventionally (Magli *et al.*, 2008; Mansour *et al.*, 2011). The 4–8 cell embryos were transferred on Day 3, after oocyte pick up. One hundred mg/day of progesterone in oil was administered intramuscularly as luteal-phase support.

We hypothesized that the hCG injection into the uterine cavity after oocyte retrieval would enhance the endometrial receptivity by triggering implantation-pathway gene expression.

Participants were asked to perform a pregnancy test at 2 weeks after ET, and if pregnant, TVSs at 3, 4 and 10 weeks after ET were performed to detect biochemical pregnancy, gestational sac, fetal cardiac activity and ongoing pregnancy, respectively. Then, the pregnant participants were introduced to a prenatal care clinic. At ~14 weeks of pregnancy, we called up the patients and asked them if they were still pregnant. Our primary outcomes were biochemical pregnancy, clinical pregnancy and implantation rates. Our secondary outcomes were ongoing pregnancy, abortion and ectopic pregnancy rates. Biochemical pregnancy was defined by a beta-hCG rise at 2 weeks after ET. Clinical pregnancy was defined by the detection of fetal cardiac activity at TVS. Implantation rate was defined by the number of gestational sacs detected by the first TVS after ET per number of embryos transferred. Ongoing pregnancy rate was defined by a pregnancy of over 14 weeks. The abortion rate was defined by pregnancy loss before 20 weeks after ET.

A clinically significant increase in implantation was considered to be 20% (from 15% to 35%), and with a power of 80% and significance level of 5%, a total of 150 women were needed. Accounting for a 7% dropout rate, an allocation of 80 women in each study group was considered as appropriate. A computer-generated randomization list with a block size of 4 with 1:1 allocation was used to randomize patients. The treatment allocation was placed in a sealed, opaque envelope and picked up consecutively by an operating room technician during the oocyte retrieval procedure. She was the person who was aware of the patient's allocation, and she also prepared and handed the intervention drug to the physician. The patient, the physician and the biomedical statistician were blinded to the group assignment of the participants.

Statistical analyses

Data were presented as means (SD) and frequencies (%) for numeric and categorical variables, respectively. The assumption of normality or the distribution of numeric variables was assessed by the Kolmogorov–Smirnov test. Independent *t*-test and Mann–Whitney *U* test were used for normally and non-normally distributed variables, respectively. The proportions of desired outcomes (primary and secondary) were compared by Fisher's exact test in the two groups. Also, odds ratios (ORs) and risk differences (RDs) and their 95% CIs were computed as relative and absolute effect sizes. In all analyses, $P < 0.05$ was considered as statistically significant (Pocock, 2013). No attempt was made to impute missing data occurred by dropouts. The analyses were done on a per protocol basis. Analyses were performed by SPSS 16 software.

Results

A flow chart of patient recruitment is shown in Fig. 1. There was no statistically significant difference in demographic and baseline clinical characteristics of participants (Table I). The biochemical pregnancy rate, 42/71 of the participants in the hCG group versus 21/67 of the participants in the control group (59.2 versus 31.3%; $P = 0.001$; OR = 1.88; 95% CI, 1.26–2.82; RD = 27.8; 95% CI, 11.2–42.3), the clinical pregnancy rate, 36/71 of participants in hCG group versus 11/67 of participants in control group (50.7 versus 16.4%; $P < 0.001$; OR = 3.08; 95% CI, 1.71–5.55), and the implantation rate (37% versus 17%; $P = 0.012$; OR = 2.29; 95% CI, 1.02–5.14) were statistically greater in the hCG group, compared with the control (Table II). Pregnancy rates in first cycle IVF cases are compared in Table III.

In the hCG group compared with the control group, the abortion and ectopic pregnancy rates were not statistically different. There was only one ectopic pregnancy that was in the control group (Table II). There were no adverse events observed in the study groups.

Because of the three correlated primary outcomes and added false-positive results, the Bonferroni-adjusted α level (Fleiss, 2011) was calculated equal to 0.017 (0.05/3) and based on this level of significance, all of the differences in primary outcomes would still be statistically significant ($P < 0.017$) (Table II).

Discussion

In this study, we demonstrated that injecting 500 IU hCG in normal saline into the uterine cavity after oocyte retrieval significantly improved the implantation and pregnancy rates in antagonist cycles with ICSI and fresh ET. The procedure is simple and practical, because it is performed immediately after oocyte retrieval under general anaesthesia, without additional stress and inconvenience to the patient.

The beneficial effect of hCG on the endometrial receptivity and its role on the embryo-endometrial crosstalk to promote implantation is mentioned in multiple studies (Licht *et al.*, 2007; Mansour *et al.*, 2011; Bourdiec *et al.*, 2013a; Strug *et al.*, 2016). HCG is one of the first paracrine factors secreted by cleavage-stage embryos, and its role for inducing gene expression cascade towards implantation is proposed by several authors (Banerjee and Fazleabas, 2011; Cole, 2012; Bourdiec *et al.*, 2013b).

Hyperglycosylated hCG, secreted by cytotrophoblast cells, is the principal source of early pregnancy hCG. It causes cytotrophoblast growth and differentiation, increases the capacity of apical cell adhesion, prevents apoptosis and provokes the invasion of the endometrium by metalloproteinases. It increases natural killer cells, inhibits macrophage migration, antagonizes transforming growth factor beta

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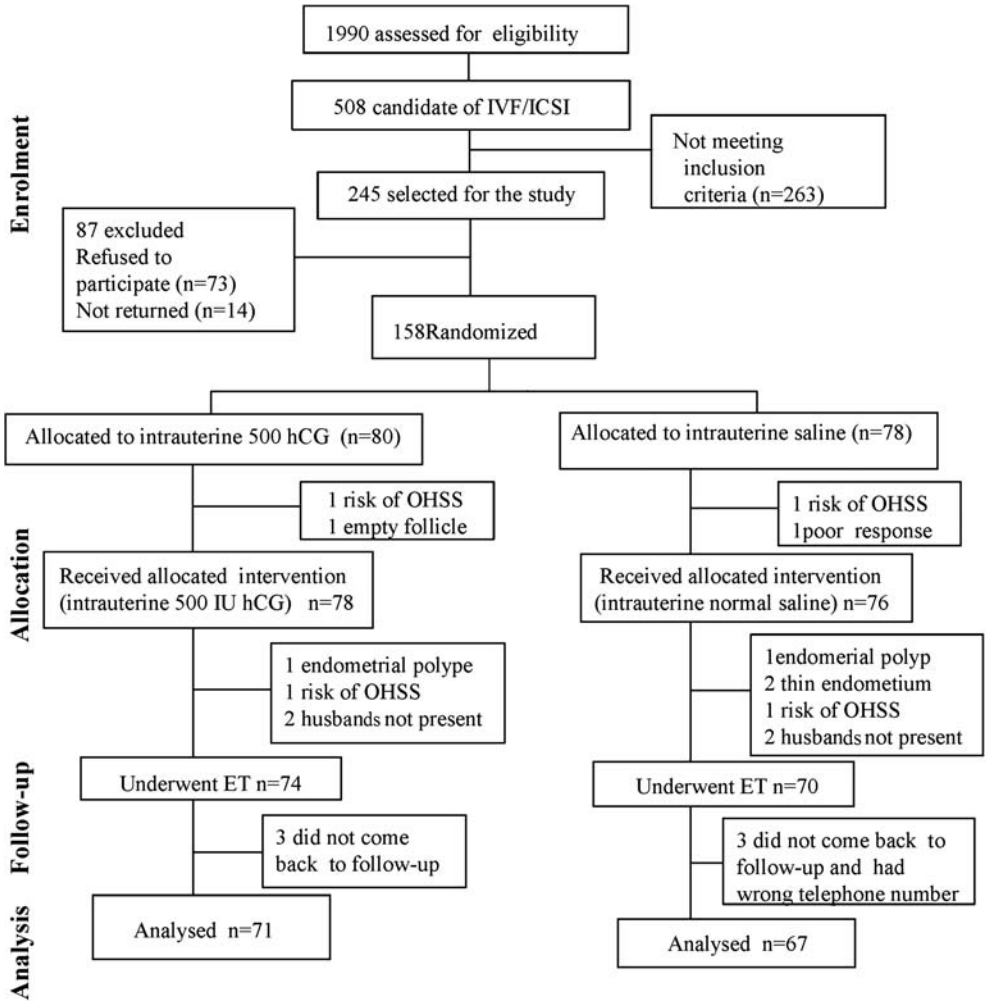

Figure 1 CONSORT flow diagram of patient selection for randomized clinical trial (RCT) of intrauterine administration of 500 IU hCG immediately after oocyte retrieval on the outcome of ICSI and fresh embryo transfer (ET).

and so modulates maternal immune response, promotes implantation and establishes a pregnancy (Bourdiec *et al.*, 2013b). hCG plays an important role in the proliferation of myometrial smooth muscle cells, reduces cell contractility and increases progesterone receptors. The hCG/LH receptor genes have been shown in human sperm, Fallopian tube and uterine spiral arteries, suggesting a role for them in reproduction (Cole, 2010, 2012).

Strug *et al.* (2016) demonstrated that *ACTA2* and sub-epithelial and perivascular α-smooth muscle actin expression, critical in preventing apoptosis and the regulation of the decidual marker IGFBP-1, important in endometrial receptivity, was induced by intrauterine hCG. Licht *et al.* (2007) have also shown that the inhibition of IGFBP-1, which extends the window of receptivity, is induced by intrauterine infusion of hCG, that will overcome the luteal-phase defect encountered in fresh cycles of ovarian stimulation. NOTCH-1 (a member of the NOTCH transmembrane receptors family) protein expression, an important determinant of cell survival or fate, required to maintain endometrial integrity and C3 (which may serve to prime the immune response for pregnancy) are targets of intrauterine hCG (Strug *et al.*, 2016). Beneficial effects of intrauterine administration of hCG before cleavage-stage ET on the outcome of IVF/ICSI cycles have been reported by many authors (Mansour *et al.*, 2011; Santibañez *et al.*, 2014; Zarei *et al.*, 2014). This beneficial effect has not been demonstrated for intrauterine hCG administration before blastocyst stage ET (Hong *et al.*, 2014; Wirleitner *et al.*, 2015). The drug probably needs more time to effect the endometrium (Licht *et al.*, 2007; Hong *et al.*, 2014) and to reduce the uterine dyssynchrony and advancement provoked by ovarian stimulations in ART cycles (Strug *et al.*, 2016).

Wirleitner *et al.* (2015) studied the intrauterine administration of 500 IU hCG in 40 µl culture media, 2 days and immediately before blastocyst transfer. They did not notice any beneficial effect for clinical outcomes in blastocyst transfer cycles. Some points remain to be elucidated in their study. They injected hCG 0.5 cm below the fundus, but they did not mention at which distance from the fundus they had transferred the blastocysts. Typically, ET is done much lower, ~1 to 2 cm below the fundus (Schoolcraft, 2016). However, it is difficult to adjust ET to the same place after 2 days. The other probable reason of ineffectiveness of Day 3 hCG administration in the Wirleitner *et al.* study

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Table I Baseline characteristics of participants in the intrauterine 500 IU hCG group, compared with the control group.[a]

	hCG group ($n = 71$)	Control group ($n = 67$)
Age (y)	30.6 (7.1)	32 (5.9)
BMI (kg/m^2)	27.6 (5.1)	27.3 (5.6)
Infertility duration (y)	8 (1.6)	7.5 (0.9)
Oestradiol level (pg/ml)[b]	1771.8 (203.5)	2096.6 (161.1)
Progesterone level (ng/ml)[b]	0.9 (0.1)	1.1 (0.2)
AMH (ng/ml)	1.6 (0.2)	2.1 (0.3)
Endometrial thickness (mm)[c]	8.7 (1.2)	8.6 (1.2)
Retrieved oocytes (*n*)	11 (0.6)	12.4 (0.6)
M2 oocytes (*n*)	9.3 (0.6)	10.1 (0.6)
2pn oocytes (*n*)	6.9 (1.2)	6.1 (0.3)
High-quality embryos (*n*)[d]	3.1 (0.3)	3.7 (0.5)
Fertilization rate (%)	0.6 (0.1)	0.6 (0.1)
Transferred embryos (*n*)	2.5 (0.8)	2.7 (0.8)
Transferred high-quality embryos[d]	0.8 (0.1)	0.8 (0.1)
Number of prior IVF cycles, n (%)		
0	36 (51)	35 (52)
≥1	35 (49)	32 (48)
Cause of infertility, n (%)		
Mixed female and male factors	30 (42)	28 (42)
Male factor	15 (21)	16 (24)
Tubal factor	8 (11)	7 (10)
Ovulatory dysfunction	4 (6)	3 (5)
Endometriosis	6 (9)	3 (5)
Unexplained	8 (11)	10 (15)

[a]Y, years; *n*, number. Data are mean (SD) unless stated otherwise.
[b]Peak level at trigger day.
[c]Measured at the day of oocyte pick up.
[d]High-quality embryos were defined based on the Istanbul consensus as Grade 1 or 2 and rated Good or Fair (<25% fragmentation, stage-specific cell size for the majority of cells, no evidence of multinucleation) (Executive, 2011)

is the lack of the continuous production of hCG by the embryo (blastocyst) to sustain the hCG action from Days 3 to 5 (Strug *et al.*, 2016).

The timing of the intrauterine hCG injection in our study was several hours before the physiological hCG secretion by the embryo. Considering the beginning of local hCG production by luteal-phase endometrium (Bourdiec *et al.*, 2013b), we designed this study to simulate the physiological state. We used 500 IU hCG in 0.5 ml normal saline for intrauterine injections. This volume of injectable solution is much greater than that used in other studies (20–40 µl) (Mansour *et al.*, 2011; Hong *et al.*, 2014; Wirleitner *et al.*, 2015). They chose to inject a much lower volume for intrauterine hCG, because they had to transfer the embryo a few minutes after the intrauterine hCG injection, taking into account that the high volume intrauterine injections might wash out the transferred embryos. Our selected volume for intrauterine injection is the same as in the Zarei *et al.* (2014) study. We administered intrauterine hCG in the early luteal phase, 3 days before ET, and assumed that by administering this volume, not only more of the endometrial surface would receive the drug, but also the drug would have time to provoke the pathway towards, receptivity. It was not necessary to precisely adjust the place of ET with the location of the intrauterine hCG injection. Its maximum effect on the prolongation of the implantation window by the modulation of IGFBP-1 (very useful in fresh cycles), and provoking the pathway toward implantation, would have been achieved at 48 h after its administration (Licht *et al.*, 2007; Strug *et al.*, 2016). It is assumed that the effect of intrauterine hCG administered at the time of ovarian retrieval would be continued by the hCG secreted by the transferred embryos from Day 3 onward (Licht *et al.*, 2007; Strug *et al.*, 2016). Normal saline used in the control and intervention groups to augment the volume will be absorbed after 3 days.

Insertion of an intrauterine insemination catheter and the injection of a small amount of saline into the uterine cavity (without hCG) may also have some impact on implantation. This effect could be studied by comparing this intervention with another study group without any intrauterine injection. Altogether, intrauterine saline injection in our study seems to have no effect on pregnancy rate, because the outcomes of the control group are the same as our routine centre outcomes for the same age group (Table II).

Table II Primary and secondary outcomes in the intrauterine 500 IU hCG group compared with the control group.[a]

	hCG group ($n = 71$), *n* (%)	Control group ($n = 67$), *n* (%)	OR (95% CI)	RD% (95% CI)	*P*-value[b]
Primary outcomes					
Chemical pregnancy rate	42 (59)	21 (31)	1.88 (1.26–2.82)	28 (11.2–42.3)	0.001
Fetal cardiac activity	36 (51)	11 (16)	3.08 (1.71–5.55)	34 (18.7–47.6)	<0.001
Implantation rate	37%	17%	2.29 (1.02–5.14)	20 (5.4–33.8)	0.012
Secondary outcomes					
Ongoing pregnancy rate[c]	29 (40)	9 (13)	3.04 (1.55–5.93)	27 (12.7–40.6)	0.001
Ectopic pregnancy	1 (1)	0	0.98 (0.95–1.01)	1 (−4.1 to 7.6)	0.514
Abortion rate	6 (9)	7 (10)	0.81 (0.28–2.28)	2 (−12.6 to 8.3)	0.562

[a]OR = odds ratio; RD = risk difference.
[b]P-value based on Fisher's exact test.
[c]Overall ongoing pregnancy rate during the study period in the centre where the study was run for the same age group was 14%.

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Table III Study outcomes in the first cycles of IVF/ICSI in the intrauterine 500 IU hCG group compared with the control group.

	hCG group ($n = 36$), n (%)	Control group ($n = 35$), n (%)	OR (95% CI)	RD% (95% CI)	P-value[a]
Chemical pregnancy rate	24 (67)	12 (34)	3.8 (1.3–11.5)	32 (10.4–54.4)	0.006
Fetal cardiac activity	20 (56)	6 (17)	6.0 (1.8–21.8)	41 (19.9–69.8)	0.001
Implantation rate	45%	29%	2.0 (1.06–3.95)	18 (2.8–32.4)	0.022
Ongoing pregnancy rate	19 (53)	6 (17)	5.0 (1.63–19.45)	39 (17.3–60.8)	0.002

[a]P-value based on Fisher's exact test.

Regarding the lengthy duration of the infertility (~8 years), overall low AMH levels, the paucity of high-quality embryos and the desire of our patients, we transferred 2–3 embryos at Day 3, which is one embryo more than the American Society for Reproductive Medicine (ASRM) recommendation for that age group. Our high-quality embryos were defined based on the Istanbul consensus as Grade 1 or 2 and rated Good or Fair (<25% fragmentation, stage-specific cell size for the majority of cells, no evidence of multinucleation) (Executive, 2011). The statistically significant differences between the outcomes of our two study groups show the beneficial effect of intrauterine hCG, specifically on the implantation of non-top embryos with low capacity of hCG secretion in fresh cycles of IVF/ICSI.

The overall low rate of ongoing pregnancy in our control group (14%) may reflect the characteristics of our patients including lengthy duration of the infertility (~8 years), overall low AMH levels and the paucity of top quality embryos, which are representative of our overall institute's attendees. Accordingly, our ongoing pregnancy rate for the same age group at the time period of the trial was the same as our control group, lower than the general practice.

Although there is no specific side effect mentioned in the literature for the intrauterine administration of hCG, it is best to be cautious about the probable side effects of this relatively new and experimental intervention. Theoretically, the local increase of growth factors and the modulation of the immune system may increase the susceptibility for local neoplasia and infection. In the present study, we did not observe any side effects, but obviously longer follow-up is needed to assess potentials hazards.

Statistical limitations

Because of the correlated primary outcomes, we had multiple testing and added false-positive rates in our tests regarding these outcomes. To accommodate with our sample size calculation and the power of the tests, we kept α level equal to 0.05. However, for the three correlated primary outcomes, the Bonferroni-adjusted α level (Fleiss, 2011) is equal to 0.017 (0.05/3) and based on this level of significance, all of the differences in primary outcomes would still be statistically significant ($P < 0.017$) (Table II).

In this RCT, we demonstrated that intrauterine administration of 500 IU of hCG during general anaesthesia after oocyte retrieval could effectively increase the implantation and pregnancy rates. The novelty of the timing of the hCG injection following ovarian retrieval in this study mandates further studies before recommending its routine use in clinical practice.

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Authors' roles

Conception and design: N.A. and A.G.; data collection and patient follow-up: N.A., L.F.; K.H., S.A. and F.J.; drafting the manuscript: N.A., F.M. and K.H.; analysis and interpretation of data: N.A., A.G., M.N., F.M. and K.H.; text reviewing: N.A., A.G., L.F., S.A., M.N., F.M., F.J. and K.H. All of the authors revised the draft and approved the final correction.

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Conflict of interest

None declared.

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