Journal of Gynecology Obstetrics and Human Reproduction

Official journal of the French College of Obstetricians and Gynecologists
Collège National des Gynécologues et Obstétriciens Français (CNGOF)
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Journal of Gynecology Obstetrics and Human Reproduction (eISSN 2468-7847) 2023 (volume 52) monthly 10 issues. **E-only journal.** See complete rates on https://www.elsevier-masson.fr/journal-of-gynecology-obstetrics-and-human-reproduction.html. Address order and payment to Elsevier Masson SAS, Service Abonnements, 65, rue Camille-Desmoulins, 92442 Issy-les-Moulineaux Cedex, France: payment by check or credit card (CB, EuroCard, MasterCard or Visa: indicate no, and expiration date); CIC, n° RIB 30066 10947 00010034501 43.

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Subscriptions - Tel.: +33 (0)1 71 16 55 99. Fax: +33 (0)1 71 16 55 77. https://www.elsevier-masson.fr/journal-of-gynecology-obstetrics-and-human-reproduction.html
Publisher - Pascal Léger. Tel.: +33 (0)1 71 16 52 12. E-mail: p.leger@elsevier.com
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Subscription conditions, Guide for Authors, the contents of each issue as well as the abstracts of the articles published in *Journal of Gynecology Obstetrics and Human Reproduction* are available on the website of the Journal: http://www.em-consulte.com/revue/jogoh

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E-only journal. eISSN : 2468-7847

Journal of Gynecology Obstetrics and Human Reproduction 52 (2023) 102691

Contents lists available at ScienceDirect

Journal of Gynecology Obstetrics and Human Reproduction

journal homepage: www.elsevier.com/locate/jogoh

Case Report

Ectopic pregnancy adjacent to iliac vessels managed successfully by minimally invasive treatment using local methotrexate injection: An extremely rare case and literature review

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ARTICLE INFO

Keywords:
Ectopic pregnancy
Pelvic pregnancy
Retroperitoneal ectopic pregnancy

ABSTRACT

A case report of successfully treated retroperitoneal ectopic pregnancy (REP) is presented. A 36-year-old woman, gravida 3, para 2, was admitted to hospital for suspected ectopic pregnancy with light vaginal bleeding and mild abdominal pain for 3 days at 45 days of gestation by the last menstrual period.Multiple transvaginal ultrasonography and two times laparoscopic probes led to the diagnosis of REP located to the iliac blood vessels closely. Eventually the patient was cured with the treatment using local methotrexate injection under real-time ultrasound guidance and systemic methotrexate administration. We also summarized another 31 cases of REP to further understand this disease, sharing them to arouse clinical attention for the diagnosis and treatment of REP timely.

Introduction

Ectopic pregnancy is the leading cause of death in women's first trimester, [1] which defined as the embryo planted outside the uterus. Retroperitoneal ectopic pregnancy (REP) refers to the gestational sac implanted in the retroperitoneal cavity of the pelvis and abdomen, and account for only 1 % of ectopic pregnancy [2-4,28]. The gestational sac of REP located closing to the large blood vessels and nerves of retroperitoneal cavity, which was easy to cause serious complications such as massive retroperitoneal hemorrhage, and the mortality was 7 times of common ectopic pregnancy [2,5,6]. Due to the low incidence of REP, there was still no recognized consensus or guidelines for diagnosis and treatment, which brings difficulties for REP's early diagnosis and appropriate treatment. This paper shares a case of REP and analyzes the relevant literature aim to provide more information for clinical practice of REP.

Case summary

A 36-year-old woman, gravida 3, para 2 for natural pregancy, presented with light vaginal bleeding and mild abdominal pain for 3 days at 45 days of gestation by the last menstrual period, No history of surgery or sexually transmitted infections and the hemodynamically was stable. On the day of admission, the serum β-human chorionic gonadotropin (β-HCG) was 7345 IU/L and the endometrial thickness was 21 mm detected by transvaginal ultrasonography, a low echo area approximately 23×18 mm without fluid in the cul-de-sac was noted in left (Fig. 1A). Next day, the β-HCG increased to 8215 IU/L and the transvaginal ultrasonography showed that the low echo area in the left was approximately 32×20 mm with no intrauterine gestational sac was visible on sonography (Fig. 1B).

Depended on the test results and physical sign of abdominal pain, the patient recived laparoscopic intervention for a diagnosis and treatment based on their informed consent. Laparoscopically visibled hemorrhagic tube in left side and we performed a left salpingectomy but the histopathological result showed no conception product was detected, the serum β-HCG levels still increasing after surgical. To rule out the possibility of intrauterine pregnancy, this patient accepted intracavitary uterine aspiration guided by ultrasound three days after postoperative, however no intraperitoneal or intrauterine pregnancy was detected on ultrasound and no abnormal tissue similar to trophoblastic content was observed in intima and the serum β-HCG levels keep increased to 18178

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https://doi.org/10.1016/j.jogoh.2023.102691
Received 21 September 2023; Received in revised form 23 October 2023; Accepted 3 November 2023
Available online 4 November 2023

IU/L. To further investigeted the location of gestational sac, we immediately contacted the experienced chief ultrasound physician and performed transvaginal ultrasound, finally found an about 27 *16 mm of low echo area with fetal bud and heartbeat located near the iliac blood vessels (Fig. 1C&D). Since the location of the gestational sac was quite specific, the patient cooperated with us to perform the second laparoscopic intervention under ultrasound guidance, during the operation, no abnormity or bleeding were observed from bilateral ovaries, right fallopian tube, mesangium, mesosalpinx, omentum, and small intestine loops, no uteroperitoneal fistula in the pelvis was found. But the intraoperative transvaginal ultrasound can clearly see the pregnancy sac near the iliac blood vessel, which is consistent with the previous ultrasound results (Figs. 2A & 3A).

Considering the high risk of vascular reupture associated with the surgical removal of pregnancy-sac mass which adjacent to the iliac vessel, the attending physician team and the patient and her family finally reached an agreement for the treatment of using local methotrexate(MTX) injection and systemic methotrexate administration. Under real-time ultrasound guidance, an 18 G puncture needle was advanced to the gestation sac with 50 mg of MTX slowly injected. Futher caution, the patient was intramusclar injected by MTX (1 mg/kg) every 2 days. Besides, periods intravenous leucovorin administration (0.1 mg/kg) was used as supplementation of folic acid against relatively common side effects of MTX regimens. The total duration of systemic treatment was 8 days. Two days after the operation, another MTX (50 mg) were local injected for β-HCG was still increasing, then the serum level of β-HCG started to decrease on the third day after the second intervention. When the patient was discharged from hospital, β-HCG dropped to 699 IU/L and the pregnancy mass was significantly reduced, there was also no fetal heart beat can be detected (Fig. 2B).The patient has no discomfort symptoms and normal biological function indicators during medication. After discharged from our hospital the HCG returned to negative in September by the telephone follow-up (Fig. 3B).

Literature review

Materials and methods

The search term "Retroperitoneal ectopic pregnancy[*all fields*]" were used to search studies published on the PubMed database in English from 1945 to 2023, including references and review articles of relevant case reports.

By browsing the title and abstract, the literature meeting the inclusion criteria of this study was initially screened, the articles that could not obtain the full text were excluded, and then re-screened by reading the full text, and finally the REP-related literature was selected.

Results

After preliminary screening and re-screening, 29 full textes of literatures were obtained excepted reviews. A total of 31 cases were reported in 29 literatures (Table 1).

The average age of 31 cases was 30.7 years. Among them, 48.4 % (15/31) has a history of fallopian tube surgery for 7 cases of right salpingectomy, 2 cases of left salpingectomy and 6 cases of bilateral salpingectomy, 54.8 %(17/31) were spontaneous pregnancy, 32.3 % (10/31) used assisted reproductive technology, 8 cases were IVF-ET, 1 case was vitro fertilization and 1 case was IUI with controlled ovarian stimulation. Abdominal pain was the most common symptom of REP (19/31,64.5 %) with 2 cases had symptoms of vaginal bleeding, 2 cases had only vaginal bleeding, 2 cases only felt pain in the left lumbar back, and 7 patients had no special symptoms other than amenorrhea.

Ultrasound was the main methods of REP's diagnostic especially transvaginal ultrasound (12/31,38.7 %), followed by CT (9/31,29.0 %) and MRI(4/31,12.9 %). 35.5 % (11/31)of patients used two or more auxiliary inspections. 1 case used 3D color doppler ultrasonography to diagnose REP. According to case statistics, most of the REP occurred in the abdominal cavity (24/31,77.4 %), and the rest in the pelvic cavity (7/31,22.6 %). The common feature of different kind of REP was the relationship with vessels, the abdominal REP is mainly located near the abdominal aorta, and the pelvic REP is mainly located in the paracentral area as the broad ligament.

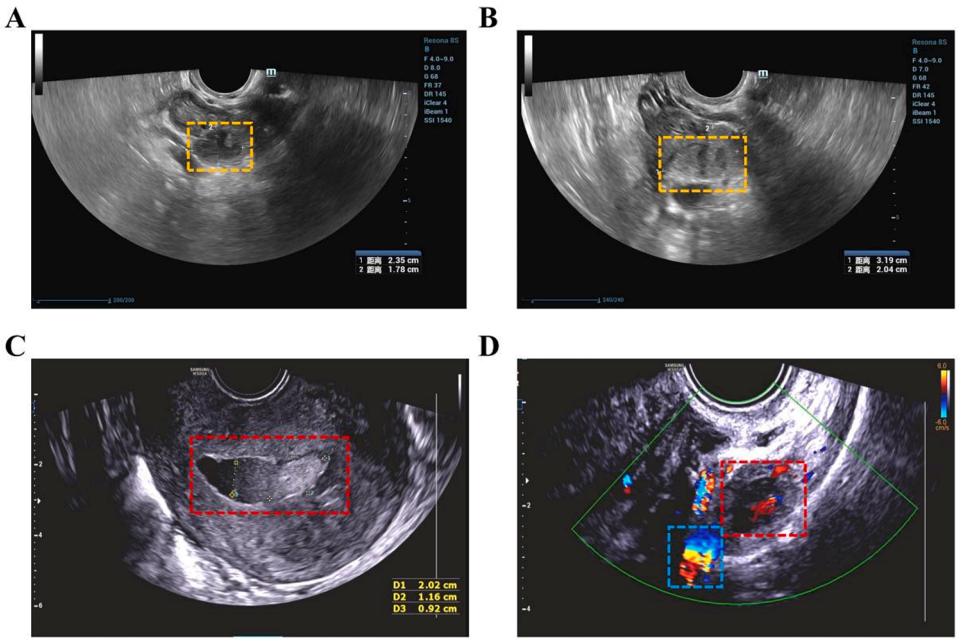

Fig. 1. A&B Transvaginal ultrasound revealed the low echo area in the left with no intrauterine gestational sac was visible on sonography on two days.(Yellow dotted box:the low echo area) C &D Transvaginal ultrasound revealed that near the iliac blood vessels the low echo area and a heartbeat(Red dotted box: gestational focus Blue dashed box: blood vessels).

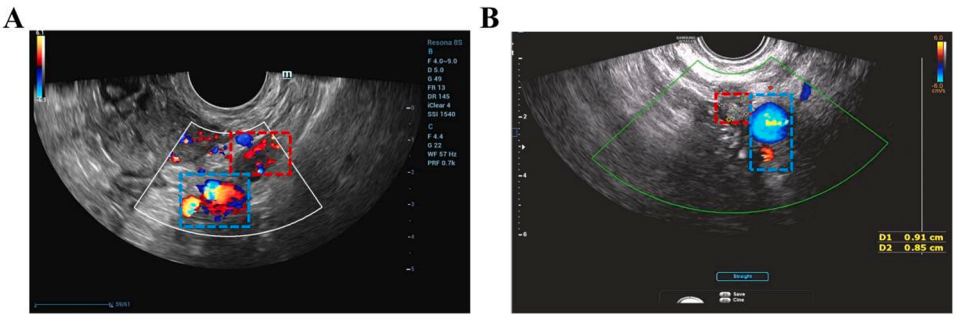

Fig. 2. A&B Reexamination of vaginal ultrasound before discharge showed that the pregnancy mass was significantly reduced and there was no fetal heart beat.(Red dotted box: gestational focus Blue dashed box: blood vessels).

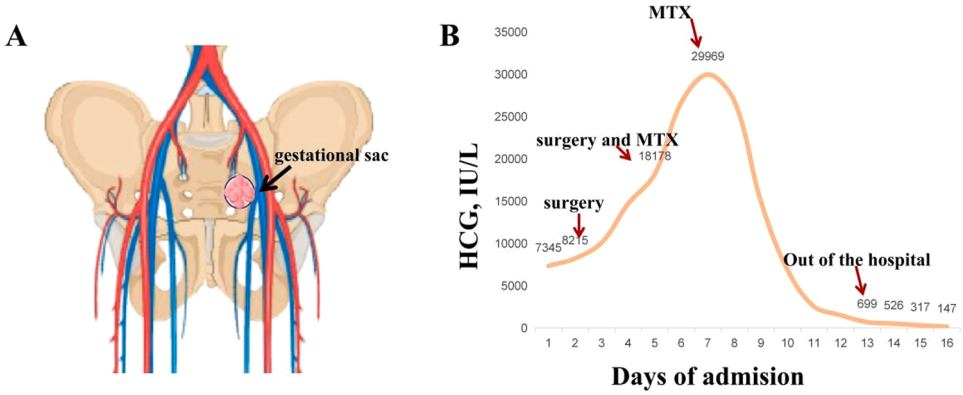

Fig. 3. A In our case, the location of the gestational sac. B The changing trend of HCG.

The surgical methods included laparoscopic surgery (14/31,45.2 %) and laparotomy (17/31,54.5 %), besides of them, 2 cases accepted laparoscopic surgery followed by laparotomy to remove pregnancy tissues, 1 case accepted robot-assisted surgical after two laparoscopic explorations, another 2 cases only locally injected MTX under CT guidance to kill the embryo successfully and one of them was misdiagnosed as choriocarcinoma and treated by MTX chemotherapy, eventually underwent the laparotomy and was cured. Experiencing 2 or more times of surgical treatment had 8 patients.

In addition, two cases mentioned that the patients had endometriosis, and two cases invited surgeons (abdominal surgery, vascular surgery) to participate in the surgery.

Discussion and comparison with existing literature

REP is an extremely rare ectopic pregnancy with no recognized guidelines or consensus for diagnosis and treatment, and many doctors lack relevant knowledge especially those in grassroots hospitals. In 1958, Williamson et al. reported the first case of REP, [7] since then there are literature reports REP increasely, however, apart from case reports and reviews, no further studies have been conducted.

Assisted reproductive technology may increase the risk of a particular type of ectopic pregnancy, [8] but the majority of cases we counted were spontaneous pregnancy (17/31,54.8 %). Interestingly, there seems to be a certain relationship between salpingectomy and the occurrence of REP, almost a general number of patients have a history of salpingectomy (15/31,48.4 %), which provides certain ideas for the occurrence mechanism of REP. But there were still 16 cases that explicitly mentioned that the patient had no history of pelvic or abdominal surgery. Therefore, regardless of whether the patient has a history of pelvic or abdominal surgery, the occurrence of REP should be vigilant.

The pathogenesis of REP is currently unclear and most likely multifactorial, and three theoretical hypotheses have been proposed: 1. In the case of assisted reproduction, retrograde migration or uterine perforation occurs after embryo transfer (e.g. pore in the uterine horn after salpingectomy). A total of 10 cases in our review acceptedassisted reproductive technology, and 9 of them had a history of tubal surgery, which provided certain supporting evidence for this theoretical mechanism. 2. Trophoblast cells or embryo sacs may migrate along blood vessels or lymphatics. In all the cases we collected, the REP was located near the retroperitoneal great blood vessel and 77.4 % near the abdominal aorta. In addition, the video article by Yuan et al. detected the presence of lymphatic tissue around pregnancy through pathology. These evidences support the hypothesis of vascular or lymphatic migration. 3. There may be congenital or acquired defects in the peritoneum or sinus tract, through which trophoblast cells invade the retroperitoneum. The cases we collected did not explicitly report the presence of obvious peritoneal defects or fistulas observed during the operation, but there are 2 patients had endometriosis which may cause peritoneal defects.

The diagnosis and treatment of REP can be quite challenging. Abdominal pain (19/31,64.5 %) was the main symptom of REP and was not specific enough to differentiate it from the common ectopic pregnancy. Ultrasound, CT, and MRI are the main diagnostic methods for REP, especially ultrasound which can detect embryo or fetal heart. REP occurs in a wide range of sites, which requires that when considering the possibility of ectopic pregnancy, the ultrasound scan should be as comprehensive as possible to include all parts of the pelvic and abdominal cavity, especially near the big blood vessels, rather than just the uterus and paratuberterine. In our case, the gestational sac was near the pelvic floor iliac blood vessels, which could easily be diagnosed as an adjoint ectopic pregnancy. There are 2 cases that treating REP successful only by locally injecting of MTX, but this method has disadvantages such as long treatment time and uncertain efficacy. Therefore surgical removal of pregnancy mass is still the main method to cure REP, and

Table 1
Information on all reported retroperitoneal ectopic pregnancy patients.

First author, Year	Age (years)	Fallopian tube surgery	Pregnancy way	Symptoms	Auxiliary inspection	Site of REP	Operation method	Use of MTX	Remove pregnancy tissues
Williamson,1958 [4]	28	NM	NM	Vaginal bleeding and abdominal pain	NM	The broad ligament	Laparotomy	No	Yes
Hall,1973 [6]	21	Right salpingectomy	NM	Vaginal bleeding and abdominal pain	NM	Above the bifurcation of the aorta	Laparotomy	No	Yes
Cordero,1994 [7]	26	NM	NM	Abdominal pain	NM	Left broad ligament	Laparotomy	No	Yes
Cordero,1994 [7]	39	NM	NM	Abdominal pain	NM	Right broad ligament	Laparotomy	No	Yes
Dmowski,2002 [8]	34	Bilateral salpingectomy	IVF-ET	Abdominal pain	TAS	Attached to portal veins	Laparotomy	No	Yes
Reid,2003 [9]	28	Bilateral salpingectomy	Vitro fertilization	Abdominal pain	NM	Bifurcation of the iliac artery	Laparoscopic	No	Yes
Lee,2005 [10]	21	No	Spontaneous pregnancy	Abdominal pain	TAS	Left paraaortic region	Laparotomy	No	Yes
First author, Year	**Age (years)**	**Fallopian tube surgery**	**Pregnancy way**	**Symptoms**	**Auxiliary inspection**	**Site of REP**	**Operation method**	**Use of MTX**	**Remove pregnancy tissue**
Chang,2008 [11]	33	No	Spontaneous pregnancy	Abdominal pain and syncope	TVS	Left paracolic sulcus	Laparoscopic	No	Yes
Bae,2009 [12]	28	No	Spontaneous pregnancy	Vaginal bleeding	TVS, TAS and CT	Anterior aspect of the IVC	Laparoscopic*2	No	Yes
Persson,2010 [13]	33	Right salpingectomy	IVF-ET	Vaginal bleeding	3D color Doppler ultrasoNography	Ileopsoas muscle under the external iliac vein	Laparoscopic*2 and robot-assisted surgical	No	Yes
Srboljub,2010 [14]	36	No	Spontaneous pregnancy	Abdominal pain	B-mode ultrasound	Right broad ligament	Laparotomy	No	Yes
Okorie,2010 [15]	28	No	Spontaneous pregnancy	Abdominal pain	TAS	Right of abdominal aorta	Laparotomy*2	Yes	Yes
Alicia,2011 [16]	37	No	IUI with controlled ovarian stimulation	Abdominal pain	TVS	Left uterosacral ligament	Laparoscopic	Yes	Yes
Jiang,2014 [17]	33	Right salpingectomy	Spontaneous pregnancy	Abdominal pain	TAS, TVS, MRI and CT	Near the abdominal aorta	Laparotomy	Yes	Yes
First author, Year	**Age (years)**	**Fallopian tube surgery**	**Pregnancy way**	**Symptoms**	**Auxiliary inspection**	**Site of REP**	**Operation method**	**Use of MTX**	**Remove pregnancy tissues**
Liang,2014 [18]	26	Left salpingectomy	IVF-ET	pain in the left lumbar back	TAS and CT	Left side of abdominal aorta	Laparoscopic and laparotomy	No	Yes
Ouassour,2017 [19]	35	Left salpingectomy	Spontaneous pregnancy	Amenorrhea	TVS, TAS and MRI	Left side of abdominal aorta	Laparotomy*2	No	Yes
Yang,2017 [20]	42	No	Spontaneous pregnancy	Abdominal pain	B-mode ultrasound	Next to the lower edge of the Left broad ligament	Laparoscopic	No	Yes
Zhang,2018 [21]	29	No	Spontaneous pregnancy	Abdominal pain	TVS	Left side of the abdominal aorta	Laparotomy	Yes	Yes
Pak,2018 [22]	30	No	Spontaneous pregnancy	Abdominal pain	TAS	Left-sided retroperitoneal (kidneys)	Laparotomy*2	No	Yes
Lu,2018 [23]	31	Right salpingectomy	Spontaneous pregnancy	Vaginal bleeding and abdominal pain	TVS	Adjacent to abdominal aorta and inferior vena cava	Laparoscopic	No	Yes
First author, Year	**Age (years)**	**Fallopian tube surgery**	**Pregnancy way**	**Symptoms**	**Auxiliary inspection**	**Site of REP**	**Operation method**	**Use of MTX**	**Remove pregnancy tissues**
Huang,2019 [24]	31	Fenestration of the Right fallopian tube	Spontaneous pregnancy	Amenorrhea	B-mode ultrasound and CT	Between the abdominal aorta and inferior vena cava	No	Yes	No
Huang,2019 [7]	37	Bilateral salpingectomy	IVF-ET	Amenorrhea	B-mode ultrasound and CT	Left side of the abdominal aorta	No	Yes	No
Le,2020 [25]	31	Bilateral salpingectomy	IVF-ET	Abdominal pain	TVS and CT	Left side of the abdominal aorta	Laparotomy	No	Yes

(*continued on next page*)

Table 1 (*continued*)

First author, Year	Age (years)	Fallopian tube surgery	Pregnancy way	Symptoms	Auxiliary inspection	Site of REP	Operation method	Use of MTX	Remove pregnancy tissues
Wang,2020 [26]	33	Bilateral salpingectomy	IVF-ET	pain in the left lumbar back	B-mode ultrasound and CT	The Left psoas major muscle	Laparotomy	Yes	Yes
Wen,2021 [27]	28	No (cesarean section)	Spontaneous pregnancy	Abdominal pain	B-mode ultrasound and MRI	The abdominal aorta	Laparoscopic	Yes	Yes
Hou,2021 [28]	29	No	Spontaneous pregnancy	Amenorrhea	CT	Between abdominal aorta and left iliac artery	Laparoscopic and laparotomy	No	Yes
First author, Year	**Age (years)**	**Fallopian tube surgery**	**Pregnancy way**	**Symptoms**	**Auxiliary inspection**	**Site of REP**	**Operation method**	**Use of MTX**	**Remove pregnancy tissues**
Lorenzo,2021 [29] (Video articles)	33	No	Spontaneous pregnancy	Abdominal pain	TVS	The Left posterior parametrium	Laparoscopic*2	Yes	Yes
ZM,2022 [30] (letters)	28	Right salpingectomy	Spontaneous pregnancy	Abdominal pain	TVS	The Left side of the aorta	Laparoscopic	No	Yes
Xu,2022 [31]	29	No	Spontaneous pregnancy	Abdominal pain	TVS,TAS and CT	Adjacent to the inferior vena cava and the abdominal aorta	Laparotomy	Yes	Yes
Yuan,2022 [32] (Video articles)	32	Right salpingectomy	IVF-ET	Amenorrhea	B-mode ultrasound and MRI	Between the aorta and inferior vena cava	Laparoscopic	No	Yes
Liu,2023 [33]	27	Bilateral salpingectomy	IVF-ET	Amenorrhea	TVS and TAS	Right of abdominal aorta	Laparoscopic with real-time TAS	No	Yes

IVF-ET:in vitro fertilization and embryo transfer;TVS:transvaginal ultrasonography;TAS:transabdominal ultrasound;CT:computer tomography;. MRI:Magnetic resonance imaging;MTX:methotrexate;NM:not mentioned.

most patients had a good prognosis without MTX treatment after surgery. Recent years, reports of laparoscopic have gradually increased, but laparotomy is still the most important method for the treatment of REP (17/31,54.5 %), and possibly in order to avoid the injury of peripheral large blood vessels during surgery. In our review, 2 cases clearly mentioned the phenomenon of the placenta implanted partly on the aorta, so multidisciplinary treatment is necessary especially combined with vascular surgeon.

Conclusion

REP is a rare ectopic pregnancy, mainly implanted in the vicinity of large blood vessels, once rupture may endanger the patient's life, but its symptoms and diagnostic methods are not specific, thus more attention should be paid to the possibility of REP in patients with history of salpingectomy and endometriosis, it is particularly important for clinicians and imaging doctors to understand REP. When REP is highly suspected, B-ultrasound, CT and MRI must be paid attention for the diagnosis and localization, and freezing rapid pathological examination should be performed during the operation if necessary. Also, multidisciplinary treatment is beneficial to the diagnosis and treatment of REP. In addition, locally injection of MTX by image guidance may be an effective treatment if REP can be accurately diagnosed before surgery. We hope to promote the further research of REP and summarize the experience of diagnosis and treatment by sharing cases, so that REP can have timely diagnosis and effective treatment.

Funding statement

This work was supported by the National Natural Science Youth Foundation of China (Grant 82202618), Youth Foundation of Wuxi Municipal Health Commission (Grant Q202122), Foundation of maternal and child research project of Wuxi (Grant FYKY202211) and Foundation of medical Education Collaborative Innovation of Jiangsu University (Grant JDY2023012).

CRediT authorship contribution statement

Jin Huang: Writing – original draft. **Xin Zuo:** Resources. **Yaoxiang Sun:** Data curation. **Xiaoyun Wu:** Formal analysis. **Hongdi Zhu:** Conceptualization. **Wei Cui:** Writing – review & editing.

Declaration of Competing Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgments

We thank the patient and her family, who agreed to allow us to publish the clinical data. The authors are also grateful for all colleagues working at the Department of Imaging Diagnosis at Yixing Hospital. All of them attributed to taking care of this patient and performing the operation.

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Journal of Gynecology Obstetrics and Human Reproduction

Official journal of the French College of Obstetricians and Gynecologists
Collège National des Gynécologues et Obstétriciens Français (CNGOF)
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Journal of Gynecology Obstetrics and Human Reproduction (eISSN 2468-7847) 2023 (volume 52) monthly 10 issues. **E-only journal.** See complete rates on https://www.elsevier-masson.fr/journal-of-gynecology-obstetrics-and-human-reproduction.html. Address order and payment to Elsevier Masson SAS, Service Abonnements, 65, rue Camille-Desmoulins, 92442 Issy-les-Moulineaux Cedex, France: payment by check or credit card (CB, EuroCard, MasterCard or Visa: indicate no, and expiration date); CIC, n° RIB 30066 10947 00010034501 43.

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Publisher - Pascal Léger. Tel.: +33 (0)1 71 16 52 12. E-mail: p.leger@elsevier.com
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Subscription conditions, Guide for Authors, the contents of each issue as well as the abstracts of the articles published in *Journal of Gynecology Obstetrics and Human Reproduction* are available on the website of the Journal: http://www.em-consulte.com/revue/jogoh

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E-only journal. eISSN : 2468-7847

Journal of Gynecology Obstetrics and Human Reproduction 52 (2023) 102663

Contents lists available at ScienceDirect

Journal of Gynecology Obstetrics and Human Reproduction

journal homepage: www.elsevier.com/locate/jogoh

Original Article

Intrauterine instillation of human chorionic gonadotropin at the time of blastocyst transfer: Systematic review and meta-analysis

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ARTICLE INFO

Keywords:
Human chorionic gonadotropin instillation
Intrauterine instillation
Embryo transfer
Fertility

ABSTRACT

Intrauterine instillation (IU) of Human Chorionic Gonadotropin (hCG) before embryo transfer (ET) has been proposed to enhance implantation success rates. This is the first meta-analysis to evaluate the effect at the blastocyst-stage. A systematic literature search was performed using Medline, Embase, Cochrane Library and Google. Randomized clinical trials (RCTs) were included. The primary outcome combined live birth rate (LBR) and ongoing pregnancy rate (OPR). The secondary outcomes were clinical pregnancy rate (CPR), implantation rate (IR) and miscarriage rate (MR). 93 citations were identified, of which there were seven eligible RCTs. 2499 participants were included in the meta-analysis; 1331 were assigned to an experimental group and 1168 were assigned to the control group. The overall effect of IU hCG instillation on LBR and OPR was not significant: risk ratio (RR) 1.00 (95% CI, 0.90–1.12). Analysis of secondary outcomes found the effect of IU hCG instillation was not significant. Analysis of the data suggests that the studies conducted have too much heterogeneity to identify whether a specific cohort may have a significant benefit. The findings of this meta-analysis demonstrate that there is insufficient evidence at present to support the use of IU hCG instillation prior to blastocyst-stage ET.

Introduction

Optimising implantation persists as a challenge in the field of reproductive medicine; implantation failure is responsible for more than 50% of pregnancy losses [18]. Implantation is a complex process, with success dependent on a number of factors aligning correctly. The two most important components are high quality embryos and a receptive endometrium. However, the multifaceted interaction between these two components is not yet fully understood.

In the early implantation period, it has been demonstrated that hCG inhibits IGFBP-1, a member of the insulin-like growth factor binding protein family. This is significant as IGFBP-1 prevents the implantation process by binding to α5β1-integrins on the cell-surface of invading trophoblasts [19]. Other actions of hCG in this implantation period include the upregulation of leukaemia inhibitory factor (LIF), vascular endothelial growth factors (VEGFs) and matrix metalloproteinase-9 (MMP-9); all factors that are essential for successful embryo attachment, placentation, trophoblast invasion, and a range of other key processes in establishing pregnancy [19].

Human chorionic gonadotropin is considered to be one of the earliest embryonic signals and its isoform βhCG is the first to be expressed by the human embryo [3]. Gene expression studies have discovered the initiation of βhCG transcription at the 2-cell and 8-cell stage blastomeres [2,17], and the secretion of βhCG into the culture media has been detected from the 2-pronuclear (2PN), one cell stage embryo throughout embryo development to the blastocyst stage [3,5,22,23,31]. Given the influence hCG has on various cell processes, it is likely that the hCG secreted by embryonic blastocyst cells directly modulates endometrial receptivity and differentiation during the process of early implantation [11,12]. The isoform hCG is recognized as the main promotor of trophoblast invasion; low levels of this isoform have been associated with inadequate implantation and pregnancy loss [6].

As a result, intrauterine instillation of hCG before embryo transfer (ET) has been proposed as an intervention to enhance implantation, and subsequently improve clinical outcomes [7]. The procedure involves intrauterine administration of hCG via an ET catheter within minutes,

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https://doi.org/10.1016/j.jogoh.2023.102663
Received 23 May 2023; Received in revised form 3 August 2023; Accepted 30 August 2023
Available online 2 September 2023

hours, or days before the ET. A number of studies have been conducted evaluating the impact of this procedure; however, the findings are inconsistent and differ with stage of embryo at transfer.

To further understand the value of this intervention, we present a meta-analysis of randomised controlled trials investigating the effects of intrauterine hCG instillation prior to blastocyst-stage embryo transfer. The aim to focus on blastocyst-stage transfers, arises from the demonstration that there are improved success rates with blastocyst-stage transfers [14], related to a more robust assessment of embryo quality at this stage. Therefore, by focusing on outcomes from IU hCG instillation with blastocyst-stage embryo transfers, this controls for cleavage stage embryos being the cause of failed implantation and furthermore is more applicable to current clinical practice of day-5/6 embryo replacement cycles. This is the first meta-analysis to focus solely on the use of IU hCG prior to blastocyst-stage transfer in assisted reproductive technology.

Methods

The meta-analysis was completed according to PRISMA guidelines. A literature search was performed using Medline, Embase, the Cochrane Library, and Google Scholar databases for relevant randomized-controlled studies until and including July 2022 to investigate the effect of intrauterine hCG instillation prior to blastocyst transfer on live birth, ongoing pregnancy, clinical pregnancy, miscarriage and implantation rates.

The following MESH search headings were used: endometrial, intrauterine, injection, instillation, perfusion, hCG, human chorionic gonadotropin, embryo transfer/ET, blastocyst transfer, fertility, infertility, ART, assisted reproductive techniques, pregnancy, birth, miscarriage, implantation, intracytoplasmic sperm injection/ICSI, and in vitro fertilization/IVF. The "related articles'' function was used to broaden the search, and all citations identified were reviewed, irrespective of language. Using these strategies, randomised controlled trials evaluating intrauterine administration of hCG versus either a placebo procedure or no intervention, around the time of blastocyst-stage embryo transfer, were included. The search strategy and included studies are shown in Fig. 1.

Data extraction

Two reviewers (L.K. and E.T.) independently extracted the data from each study. Any disagreement was judged by the third investigator (S. M.). In case of insufficient data, authors were contacted to obtain the necessary information. Quantitative data were extracted as follows: logistics (first author, year of publication, study design, study period, study country); study groups (number of IU hCG patients vs. control patients, definition of control group, type of cycle fresh/frozen, timing of hCG before transfer, dose of IU hCG); and the following fertility-related rates: live birth, pregnancy, multiple pregnancy, ongoing pregnancy, clinical pregnancy, implantation, clinical loss per transfer and miscarriage. This data is displayed in Tables 1 and 2.

Inclusion and exclusion criteria

Studies were included if they were randomized clinical trials (RCTs). The subjects in the experimental group were infertile women who underwent in vitro fertilisation and embryo transfer (IVF-ET) at the blastocyst-stage and received an intrauterine instillation of hCG before ET by means of slow intrauterine infusion. The control group consisted of infertile women who underwent IVF-ET at the blastocyst-stage with placebo or no intrauterine hCG instillation. The primary outcomes were

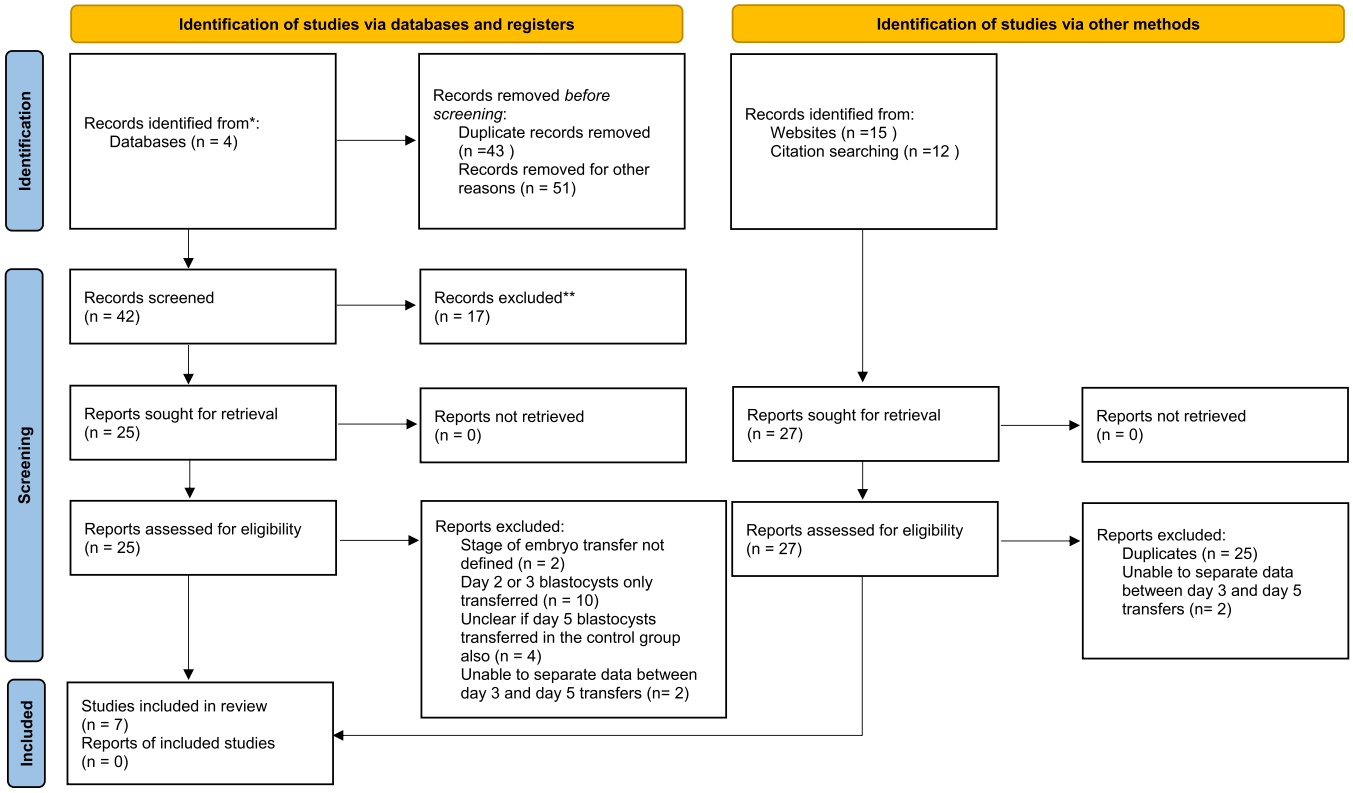


Fig. 1. PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registers and other sources *Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers). **If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools. *From:* Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/.

Table 1
Study characteristics.

Author (Year)	Study Design	Enrolment Dates	Inclusion Criteria	Study Groups	Age Range (mean ±SD)	Type of cycle (Fresh/ frozen)	Placebo infusion (control group)	Timing of hCG before transfer	Dose of hCG
Cambiaghi (2013) Brazil	RCT	January-December 2012	• Endometrial thickness >7 mm on the day the donor received hCG • At least two blastocysts on the day of ET	Total: (n = 44) Control: (n = 22) Experimental: (n = 22)	ND	Fresh	Nil *(all forwarded straight to ET)*	6 h	500 IU
Hong (2014) USA	RCT	August 2012-December 2013	• <43 years old	Total: (n = 300) Control: (n = 152) Experimental: (n = 148)	23.9–42.8 (35.1 ± 0.2)	Fresh n = 132 (44%) FET n = 168 (56%)	Culture media	3 min	500 IU *(purified-urinary placental Novarel, Ferring Pharmaceuticals)*
Wirleitner (2015a) Day 3 Czech Republic	RCT	February 2013-February 2014	• Fresh autologous blastocyst transfer on day 5 • ≤43 years old	Total: (n = 182) Control: (n = 93) Experimental: (n = 89)	22–43 (36.1 ± 4.1)	Fresh	Culture media	2 days	500 IU *(Pregnyl, ORGANON, Netherlands)*
Wirleitner (2015a) Day 5 Czech Republic	RCT	February 2013-February 2014	• Fresh autologous blastocyst transfer on day 5 • ≤43 years old	Total: (n = 1004) Control: (n = 494) Experimental: (n = 510)	20–43 (37.1 ± 4.0)	Fresh	Culture media	3 min	500 IU *(Pregnyl, ORGANON, Netherlands)*
Wirleitner (2015b) Czech Republic	RCT	ND	ND	Total: (n = 510) Control: (n = 255) Experimental: (n = 255)	38–43 Control: (40.4) Experimental: (40.3)	Fresh	Culture media	Just before	500 IU
Mostajeran (2017) Iran	RCT	September 2013-April 2014	• 20–40 years old • Infertility secondary to male factor • Regular menstrual cycle of 24–35 days • Presumed to be ovulatory	Total: (n = 94) Control: (n = 46) Experimental: (n = 48)	<40 (31.3 ± 5.2)	Fresh	Nil	10 min	700 IU *(Chorionic Gonadotropin Human, Darou Pakhsh Company, Iran)*
Liu (2019) China	RCT	January 2016-December 2016	• Repeated implantation failure (after 3 or more transfers of high quality embryos) • ≤45 years old • BMI (19–30Kg/m^2) • asal FSH<10IU/L • Normal uterine cavity on hysteroscopy • Normal maternal and paternal karyotypes • FET cycles	Total: (n = 303) Control: (n = 87) Experimental: (n = 87)	≤45 Control: (35.25±4.94) Experimental: (34.83±4.31)	Frozen	Saline	3 days	500 IU *(Choragon, Livzon Pharmaceutical Group, Inc, China)*
Abdallah (2021) Egypt	RCT	July 2018-February 2020	• 18–43 years old • Infertility scheduled for IVF with at least one good quality embryo for the following indications: unexplained infertility, male factor, ovulatory/tubal disorders	Total: (n = 181) Control: (n = 91) Experimental: (n = 90)	18–43 (31.1 ± 4.9)	Fresh n = 165 (91.2%) Frozen n = 16 (8.8%)	Culture media	4 min	500 IU *(Epifasi) (EIPICO, Tenth of Ramadan Egypt)*

Key:.
(ND) No data.
(FET) Frozen Embryo Transfer.
(RCT) Randomised Controlled Trial.
(IVF) In Vitro Fertilisation.
(FSH) Follicle Stimulating Hormone.
(BMI) Body Mass Index.
(hCG) Human Chorionic Gonadotropin Hormone.
(ET) Embryo Transfer.

Table 2
Fertility related rates.

Author (Year)	Number of embryos transferred (n) or mean (±SD)	Live Birth Rate per Embryo Transfer (%)	Pregnancy Rate per Embryo Transfer (%)	Multiple pregnancy Rate per Embryo Transfer (%)	Ongoing pregnancy Rate per Embryo Transfer (%)	Clinical pregnancy Rate following Blastocyst Transfer (%)	Implantation Rate following Blastocyst Transfer (%)	Miscarriage rate per Transfer	Miscarriage Rate per Clinical Pregnancy (%)
Cambiaghi (2013)	ND	ND	ND	ND	ND	Control: (63.3%) Experimental: (81.8%)	ND	ND	ND
Hong (2014)	Control: ($n = 240$) Experimental: ($n = 233$)	ND	ND	ND	Overall: Control: 79/152; (52.0%) Experimental: 87/148; (58.8%) Fresh cycles: Control: 40/68; (58.9%) Experimental: 44/64; (68.8%) FET cycles Control: 39/84; (46.4%) Experimental: 43/84; (51.2%)	ND	Overall: Control: 106/240; (44.2%) Experimental: 112/233; (48.1%) Fresh cycles: Control: 56/115; (48.7%) Experimental: 59/112; (52.7%) FET cycles Control: 50/125; (40.0%) Experimental: 53/121; (43.8%)	Control: 11/152; (7.2%) Experimental: 17/148; (11.5%)	ND
Wirleitner (2015a) Day 3	Control: ($n = 153$) Experimental: ($n = 144$)	Control: 34/93; (36.6%) Experimental: 31/89; (34.8%)	Control: 45/93; (48.4%) Experimental: 42/89; (47.2%)	Control: 10/93; (10.8%) Experimental: 10/89; (11.2%)	ND	Control: 37/93; (39.8%) Experimental: 33/89; (37.1%)	Control: 44/153; (28.8%) Experimental: 41/144; (28.5%)	ND	Control: 3/93; (3.2%) Experimental: 2/89; (2.2%)
Wirletner (2015a) Day 5	Control: ($n = 849$) Experimental: ($n = 868$)	Control: 198/494; (40.1%) Experimental: 188/510; (36.9%)	Control: 261/494; (52.8%) Experimental: 261/510; (51.2%)	Control: 80/494; (16.2%) Experimental: 60/510; (11.8%)	ND	Control: 228/494; (46.2%) Experimental: 213/510; (41.8%)	Control: 276/494; (55.9%) Experimental: 253/510; (49.6%)	ND	Control: 30/494; (6.1%) Experimental: 25/510; (4.9%)
Wirletner (2015b)	ND	Control: 68/225; (30.2%) Experimental: 68/255; (26.7%)	ND	ND	ND	Control: 83/225; (36.9%) Experimental: 86/255; (33.7%)	ND	Control: 15/225; (6.7%) Experimental: 18/255; (7.1%)	ND
Mostajeran (2017)	Control: (1.7 ± 0.71) Experimental: (1.4 ± 0.73)	ND	Control: 27/48; (56.2%) Experimental: 24/46; (52.1%)	ND	ND	ND	ND	ND	ND
Liu (2019)	Control: (1.33±0.47) Experimental: (1.38±0.49)	Control: 26/151; (17.2%) Experimental: 41/152; (26.9%)	ND	Control: 3/38; (7.9%) Experimental: 5/57; (8.8%)	ND	Control: 38/151; (25.2%) Experimental: 57/152; (37.5%)	Control: 39/201; (19.4%) Experimental: 61/209; (29.2%)	ND	Control: 10/38; (26.3%) Experimental: 13/57; (22.8%)
Abdallah (2021)	ND	Control: 3/19; (15.8%) Experimental: 7/24; (29.2%)	ND	ND	Control: 3/19; (15.8%) Experimental: 7/24; (29.2%)	Control: 6/19; (31.6%) Experimental: 9/24; (37.5%)	ND	Control: 3/19; (15.8%) Experimental: 2/24; (8.3%)	Control: 3/19; (15.8%) Experimental: 2/24; (8.3%)

Key:.
(ND) No data.
(FET) Frozen Embryo Transfer.

live birth rate (LBR) and ongoing pregnancy rate (OPR; defined as the number of intrauterine gestational sacs with foetal heartbeats at 12 weeks of gestation). The secondary outcomes were clinical pregnancy rate (CPR; defined as the presence of intrauterine gestational sac with positive embryonic heart activity), implantation rate (IR) and miscarriage rate (MR). Studies were excluded if pregnancy outcome incidence was not reported.

Quality assessment

The revised Cochrane risk-of-bias tool for randomized trials (RoB 2) [27] was used to assess the risk of bias in each included study. The

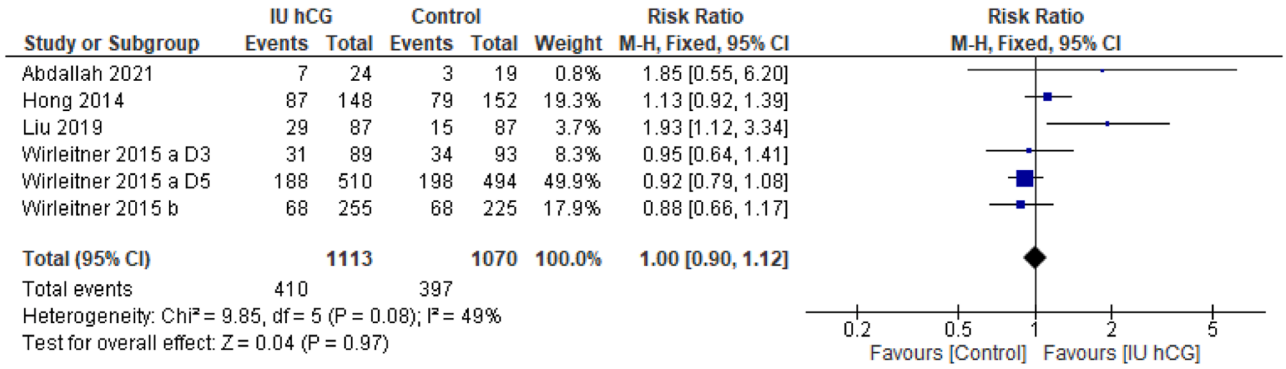
assessment domains were: risk of bias arising from the randomization process, risk of bias due to deviations from the intended interventions, risk of bias due to missing outcome data, risk of bias in measurement of outcome and risk of bias in selection of the reported result. Two investigators (L.K. and E.T.) independently evaluated the quality of each included study, and disagreements were resolved by consensus with the third investigator (S.M.).

Statistical analysis

Quantitative synthesis and subgroup analyses were conducted with the use of Review Manager version 5.4. All outcomes were dichotomous; Mantel-Haenszel risk ratios (RRs) were calculated with 95% confidence intervals (Cis) using the numbers of events in the intervention and control groups of each study.

Clinical and methodological characteristics of the included studies were examined by visual inspection of the forest-plot graphs, the overlap in confidence intervals and more formally by using the I2 statistic in order to test for statistical heterogeneity. An I2 measurement greater than 50% was taken to indicate substantial heterogeneity [15]. If heterogeneity existed (I2>50%), a random-effects model was adopted; otherwise, a fixed-effects model was applied [20].

Subgroup analyses were carried out to determine the effects of the intervention on Day 3 compared to Day 5/6 and fresh compared to frozen cycles. Results of the studies and overall analyses are shown in Figs. 2–9.



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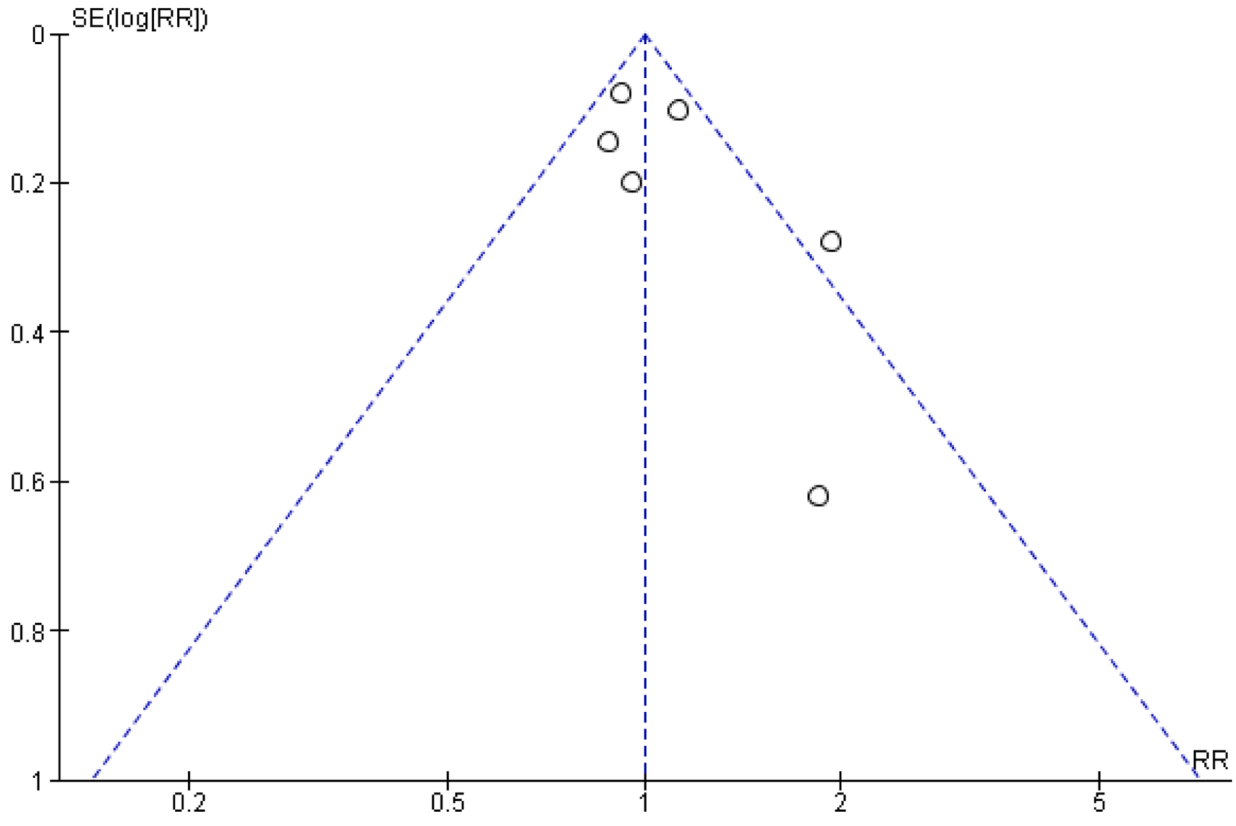


(b)

Fig. 2. Forest Plot: Combined Live Birth and Ongoing Pregnancy Rate following Blastocyst Transfer with IU hCG vs no IU hCG or placebo infusion Figure 2b. Funnel plot for Live birth/Ongoing Outcome following Blastocyst Transfer with IU hCG Intervention.

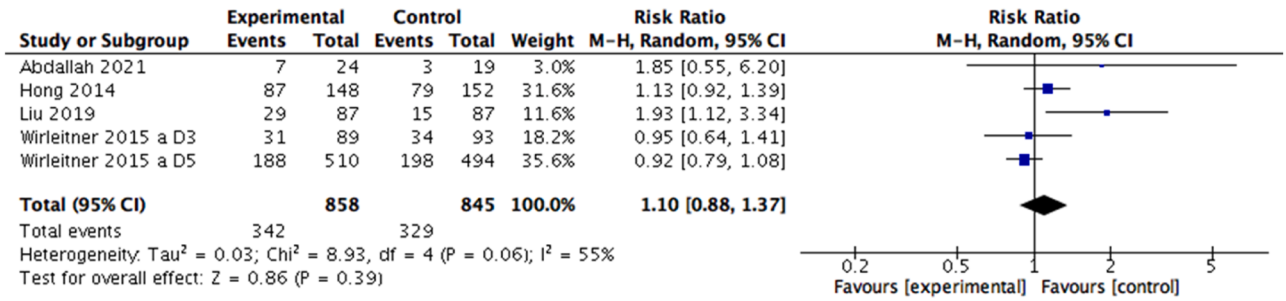


Fig. 3. Sensitivity analysis for main outcome, without abstracts.

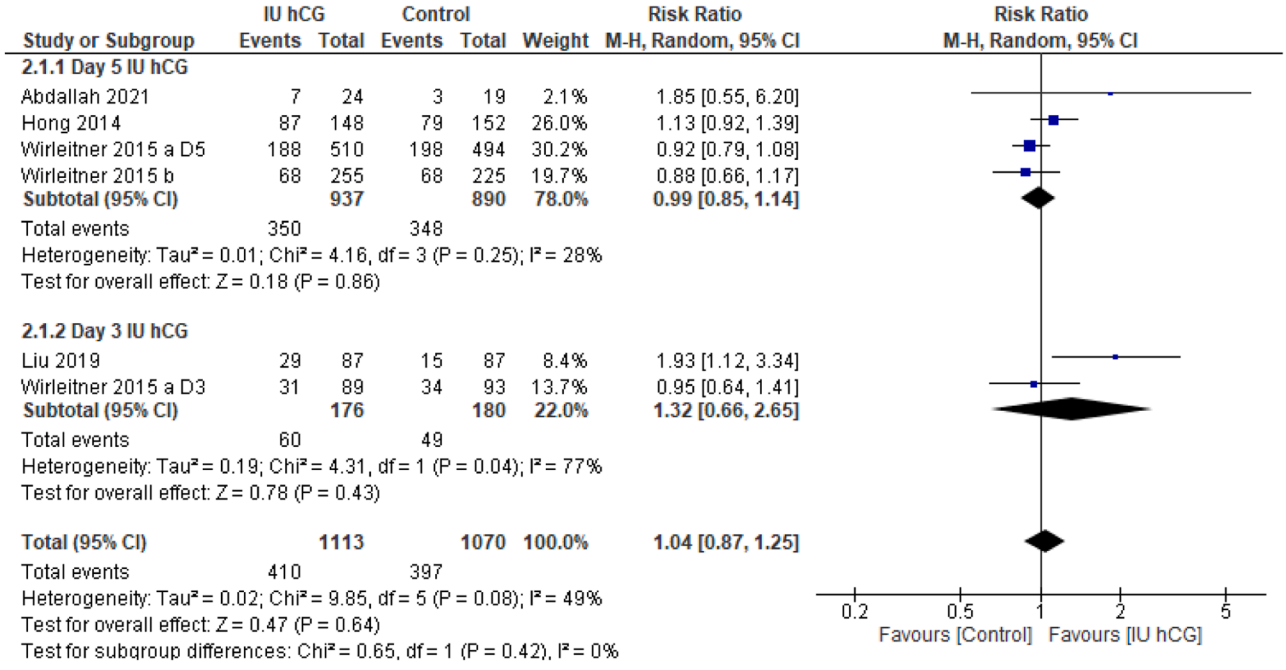


Fig. 4. Forest Plot: Combined Live birth and Ongoing Pregnancy Rate following Blastocyst Transfer with IU hCG vs no IU hCG or placebo infusion. Subgroup analysis according to the day hCG infusion was performed: Day 3 or Day of Transfer.

Results

The process of study selection followed PRISMA guidelines as outlined in Fig. 1. After excluding duplicated studies, the initial searches yielded 93 potentially relevant studies, of which seven eligible RCT studies were included in this meta-analysis [1,4,16,21,24,29,30]. Of these, two studies were published as a conference abstract [4,30]. Within this meta-analysis a total of 2499 participants were included, of which 1331 were assigned to an experimental group and 1168 were assigned to the control group. Demographic and clinical characteristics of the two groups are listed in Table 1.

The experimental group received an intrauterine instillation of hCG of 500 IU ($n = 6$) [1,4,16,21,29,30] or 700 IU ($n = 1$) [24]. The times of administration before ET varied: 'just before transfer' [30], 3 min before [16,29], 4 min before [1], 10 min before [24], 6 h before [4], 2 days before [29], 3 days before [21].

The control group differed in their treatment; placebo intrauterine infusion with culture medium occurred in four studies (2013, [1,16,29,30]), placebo intrauterine infusion with saline occurred in one study [21] and no alternative infusion instead direct to ET as per usual protocol occurred in two studies [4,24].

Of the seven studies, four studies were fresh cycles [4,24,29,30], one study used only frozen cycles [21] and two studies included both fresh and frozen cycles [1,16].

The risk of bias for all studies as per the revised Cochrane risk-of-bias tool for randomized trials (RoB 2) [27] are presented in Table 3.

Meta-analysis – primary outcome

Five of the seven studies reported on LBR or OPR per ET [1,16,21,29,30]. The incidence of this primary outcome per ET was 36.8% [410/1113] in the IU hCG intervention group and 37.1% [397/1070] in the control groups. Using the fixed effects model (as I2<50%) the overall effect of IU hCG instillation on combined LBR and OPR was shown to be not significant, with a risk ratio (RR) of 1.00 (95% CI, 0.90–1.12). For the sensitivity analysis of the primary outcomes, the data was also analysed after removal of abstracts using the random effects model (Fig 3). This further demonstrated that there was no statistical significance between the intervention and control group: RR 1.10 (95% CI, 0.88–1.37).

Subgroup analysis – primary outcome and timing of IU hCG

Subgroup analysis was performed to determine the effect of timing of the IU hCG intervention (Fig. 4). Studies were separated into two groups; group 1 referred to as 'Day 5/6 IU hCG' were those where the intervention group received the IU hCG instillation on the same day as the ET, and group 2 referred to as 'Day 3 IU hCG' were those where the

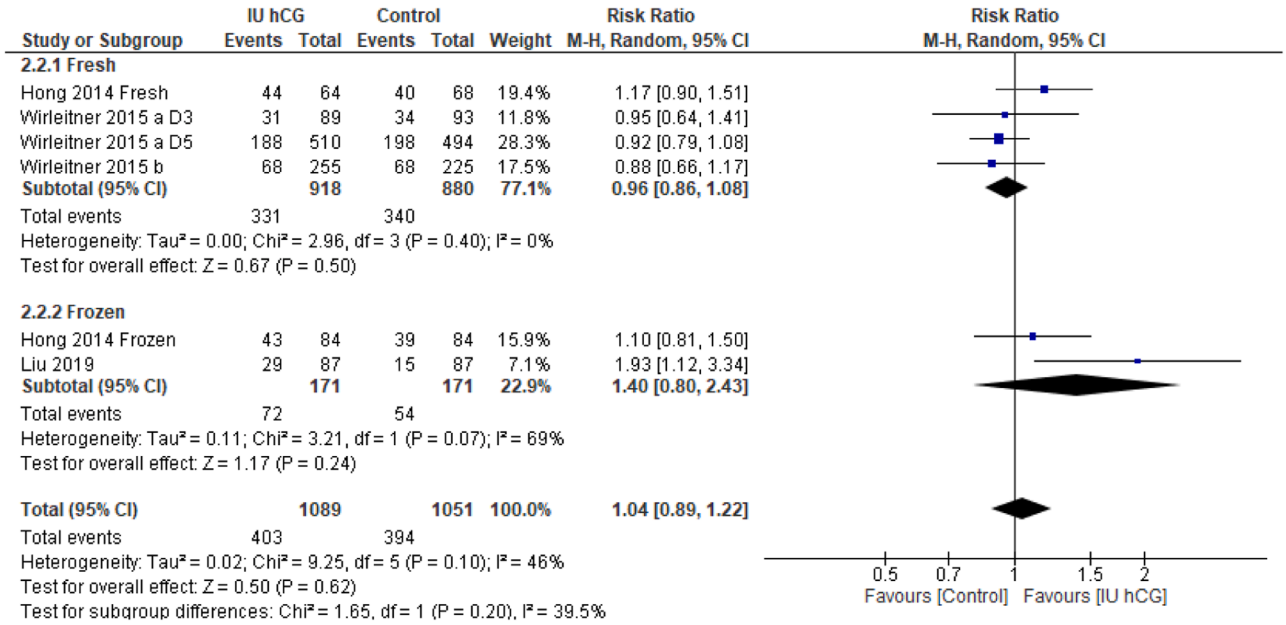


Fig. 5. Forest Plot: Combined Live birth and Ongoing Pregnancy Rate following Blastocyst Transfer with IU hCG vs no IU hCG or placebo infusion. Subgroups analysis according to whether the transfer was during a fresh cycle of during a frozen cycle.

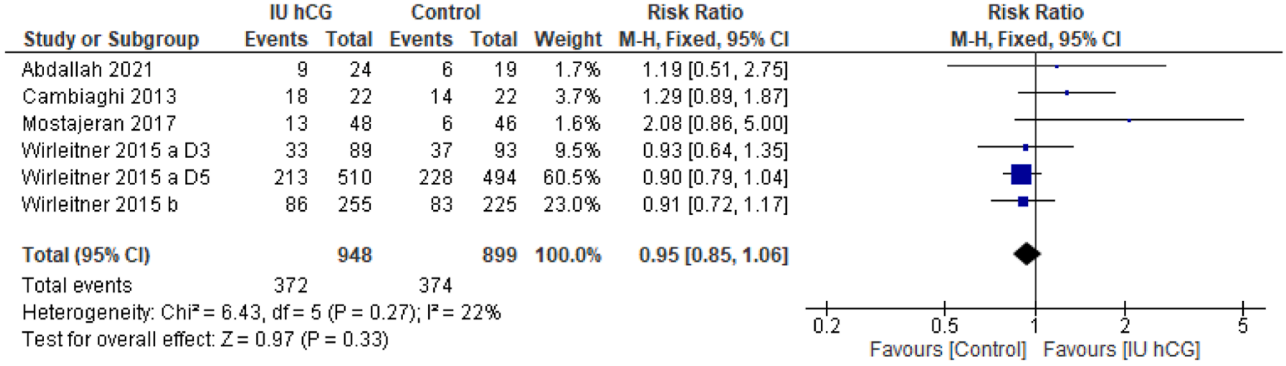


Fig. 6. Forest Plot: Clinical pregnancy rate following blastocyst transfer with IU hCG vs no IU hCG or placebo infusion.

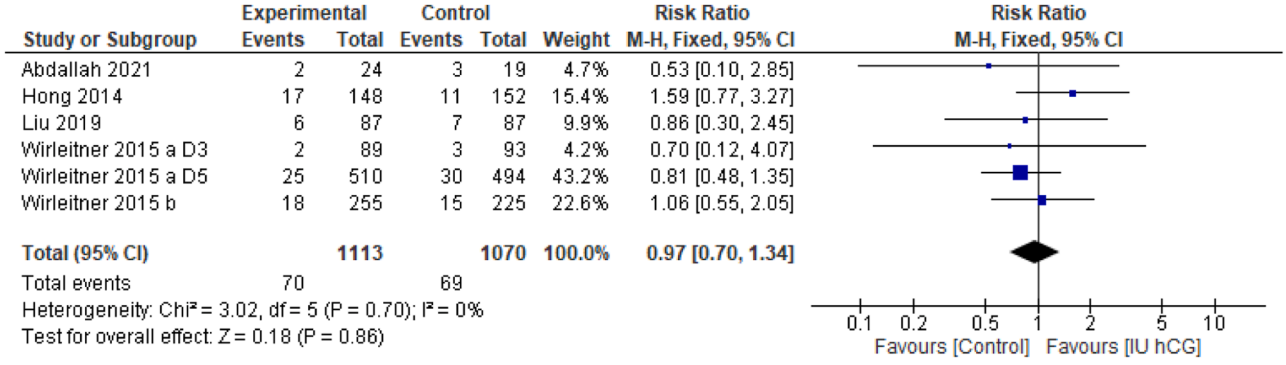


Fig. 7. Forest Plot: Miscarriage rate per transfer.

intervention group received IU hCG instillation 2–3days before the ET. The meta-analysis findings demonstrated no significant effect in the 'Day 5 IU hCG' subgroup (4 studies: [1,16,29,30]); using the fixed effects model (as I2<50%) the overall effect of D5 IU hCG instillation on combined LBR and OPR was RR 0.99 (95% CI, 0.85–1.14). The 'Day 3 IU hCG' subgroup included only two studies [21,29]; the outcomes of the analysis was a RR value of 1.32 with a confidence interval (0.66–2.65).

Subgroup analysis – primary outcome and type of cycle

Subgroup analysis was performed to determine the effect of the type of cycle (fresh or frozen) on the outcomes of IU hCG instillation (Fig. 5). Studies were separated into two groups; group 1 looked at fresh cycles [16,29,30], and group 2 looked at frozen cycles [16,21]. The meta-analysis findings demonstrated no significant effect in the fresh

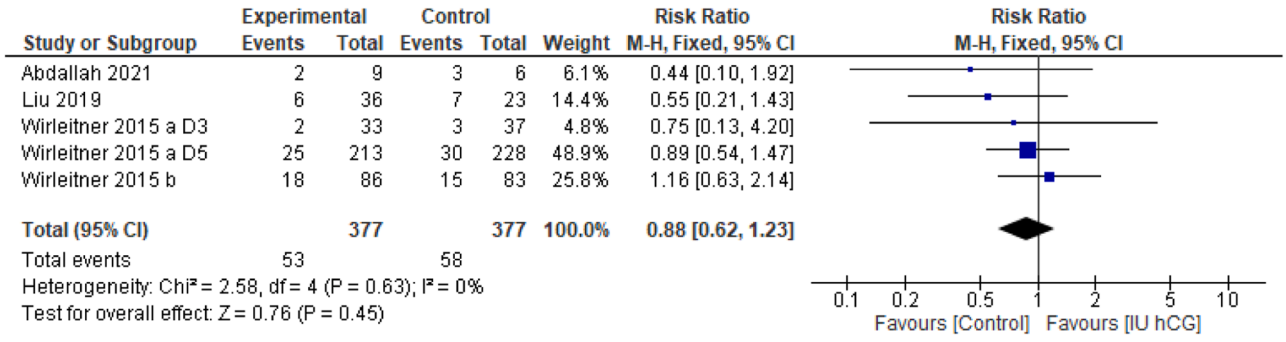


Fig. 8. Forest Plot: Miscarriage rate per clinical pregnancy.

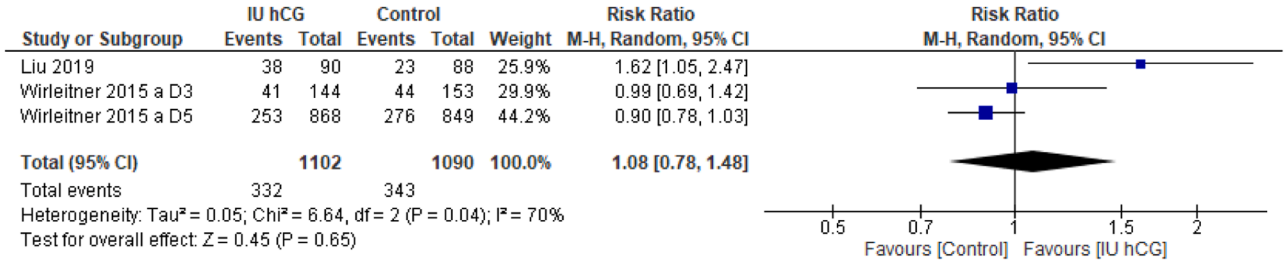


Fig. 9. Forest Plot: Implantation rate.

cycle subgroup; using the fixed effects model (as I2<50%) the overall effect of IU hCG instillation on combined LBR and OPR in fresh cycles was RR 0.96 (95% CI, 0.86–1.08). The frozen cycle subgroup included only two studies [16,21]: the outcomes of the analysis was a RR value of 1.40 with a confidence interval (0.80–2.43).

Meta-analysis – secondary outcomes

Secondary Outcome: Clinical Pregnancy Rate following Blastocyst Transfer (Fig. 6).

The CPR following blastocyst transfer of 1847 participants were included in five studies [1,4,16,24,29]. Following meta-analysis using the fixed effects model, the patients in the intervention group 39.2% [372/948] showed no significant difference to the CPR in the control group 41.6% [374/899]: the outcome of the analysis was a RR value of 0.95 (95% CI, 0.85–1.06).

Secondary Outcome: Miscarriage Rate per Transfer (Fig. 7)

The miscarriage rate following blastocyst transfer of 2183 participants were included in five studies [1,16,21,29,30]. Following meta-analysis using the fixed effects model, the patients in the intervention group 6.3% [70/1113] showed no significant difference to the miscarriage rate in the control group 6.4% [69/1070]: the outcome of the analysis was a RR value of 0.97 (95% CI, 0.70–1.34).

Secondary Outcome: Miscarriage Rate per Clinical Pregnancy (Fig. 8).

The miscarriage rate per clinical pregnancy of 754 participants were included in four studies [1,21,29,30]. Following meta-analysis using the fixed effects model, the patients in the intervention group 14.1% [53/377] showed no significant difference to the miscarriage rate per clinical pregnancy in the control group 15.4% [58/377]: the outcome of the analysis was a RR value of 0.88 (95% CI, 0.62–1.23).

Secondary Outcome: Implantation Rate (Fig. 9).

The IR following blastocyst transfer of 2192 participants were included in two studies [21,29]. Following meta-analysis using the random effects model, the patients in the intervention group 30.1% [332/1102] showed no significant difference to the IR in the control group 31.4% [343/91090]: the outcome of the analysis was a RR value of 1.08 (95% CI, 0.78–1.48).

Discussion

Main findings

This meta-analysis was conducted to determine the value of receiving an IU hCG instillation prior to blastocyst stage IVF-ET. This meta-analysis included a total of 2499 cycles across seven studies. Focusing on the primary outcomes, IU hCG instillation did not have a significant impact on LBR and OPR per ET. There was also no effect demonstrated on outcomes following subgroup analysis assessing the timing of IU hCG instillation and type of cycles (fresh versus frozen). The analysis demonstrated no effect of the intervention in fresh cycles, however, given the small sample size for frozen cycles further studies are needed to establish if IU hCG instillation affects outcomes of frozen cycle blastocyst transfers.

The results after analysis of secondary outcomes similarly demonstrated that within the identified RCTs there was no significant difference in outcomes when comparing groups with IU hCG instillation prior to blastocyst stage IVF-ET and control groups.

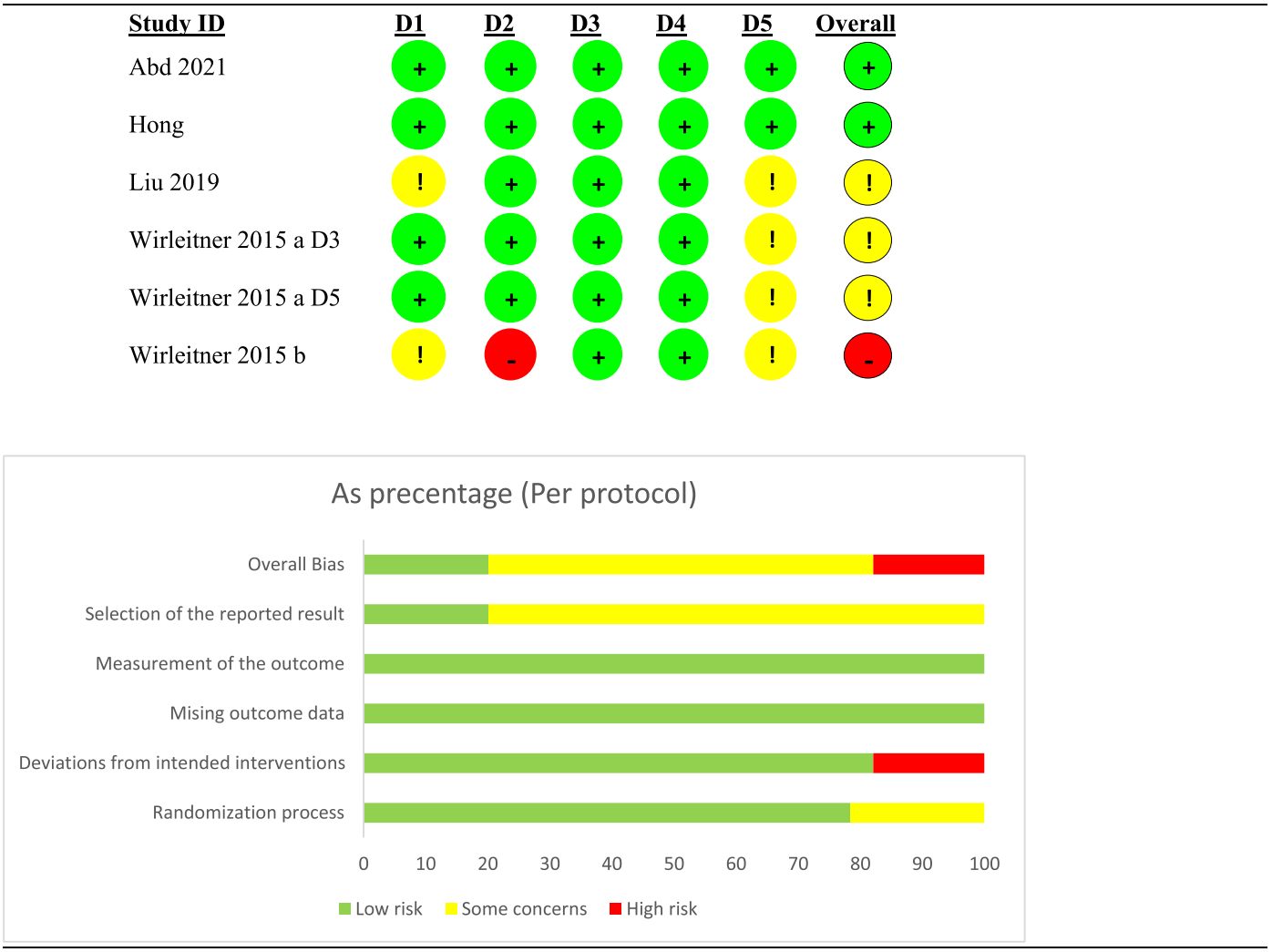
The sample size of the Day 3 IU hCG and frozen cycles, were small and the RR confidence intervals were wide, which suggests the findings may not be conclusive. Further well-designed studies are required to examine the effect of Day 3 IU hCG instillation on blastocyst-stage ET transfer and the role of IU hCG in frozen cycles of this type.

Strengths and weakness

To the best of our knowledge, this is the first meta-analysis to analyse outcomes of IU hCG instillation in the context of IVF-ET using blastocyst-stage embryos. The main strength of this study is the focus on the definition of IU hCG use at the blastocyst-stage, thus analysing a specific set of studies. The MeSH terms used for the initial search were required as keywords in the returned articles. These keywords were also searched within the title and abstract, but we accept that there is a small chance that further studies exist.

As a result of the potential variety in the protocol of IU hCG use, there is a degree of heterogeneity across the studies, which is a significant limitation. The small number of studies and heterogeneity means

Table 3
The risk of bias for all studies as per the revised Cochrane risk-of-bias tool for randomized trials for Live birth/Ongoing clinical pregnancy (RoB 2) (Sterne 2019).



subgroup analysis was limited. Sources of heterogeneity include the variation in the use of placebo with the control group, detail of luteal phase support across studies and between fresh/frozen cycles, separation of Day 5 and Day 6 ET within the definition of blastocyst transfer, inclusion criteria defining specific infertility causes, and number of embryos transferred per transfer likely to influence outcomes separately. Liu (2019) includes only patients with recurrent implantation failure (RIF), this is in contrast to other studies which includes infertile patients undergoing IVF but without RIF; this is an example of a clear source of heterogeneity. Recent guidance from ESHRE focused only on women with RIF, evaluated all available studies related to IU HCG and concluded there was insufficient data from RCTs in this cohort to support the use of IU hCG instillation in clinical practice [9].

Comparison with other studies

Several meta-analyses of RCTs [13,26,32], a Cochrane review [8] and a critical review [33] were conducted to evaluate the efficacy and usefulness of IU hCG treatment across a range of IVF-ET cases. However, these comparative studies demonstrate considerable clinical and statistical heterogeneity amongst published articles, with conflicting results. This heterogeneity is due to wide variations in study populations (donor vs non-donor, patients ages, indication for IU hCG, low number of cases etc.) and experimental design (fresh vs frozen ET, day 3 vs day 5 embryo transfer, variation in timing and dose of IU hCG.). Thus, to date there is not enough evidence to draw solid conclusions on the use of IU hCG instillation.

The Cochrane review identified the stage of ET (cleavage vs. blastocyst) as a key variable impacting outcomes [8]. The majority of studies evaluated were related to cleavage stage embryos and, according to the Cochrane summary (2018), there is moderate quality evidence that women undergoing transfer at this stage (day 2/3) using IU hCG instillation with a dose ≥500IU have an improved LBR. However, there is insufficient evidence for similar treatment benefit related to blastocyst transfer.

The meta-analysis we conducted focused solely on blastocyst-stage transfer and despite the large number of cycles, the findings demonstrated that there is insufficient evidence at present to support the use of IU hCG instillation prior to blastocyst-stage ET. It is likely that given the assessment of the data, the studies conducted have too much heterogeneity to identify whether a specific cohort of patients with a particular IU hCG protocol may have a significant benefit.

Implications for clinical practice

The concept of IU hCG instillation as a potential benefit arises from our understanding of its role in the process of implantation. Human chorionic gonadotropin is known to have a wide range of actions which enable placental, uterine and foetal development in the course of the pregnancy [7,11]. Human chorionic gonadotropin (hCG) is considered

to be a key player in regulating the foetal-maternal interface. The blastocyst has been shown to secrete hCG 6–8 days after fertilization [28,31], which locally enhances the intrauterine environment by influencing immunological tolerance and stimulating endometrial growth via its effect on endometrial stromal fibroblast. Human chorionic gonadotropin promotes the secretion of cytokines, which prolong endometrial receptivity, stimulate angiogenesis and promote endometrial remodelling to ensure the ideal environment for implantation [25]. Therefore, as the earliest embryonic product, hCG may be a key regulator in triggering the complex process of embryo implantation.

Whilst literature suggests that intrauterine hCG infusion is a safe procedure, it is important to consider the potential adverse effects of this intervention. The basis for this concern arises from clinical investigations showing that prolonged exposure to hCG may be detrimental to endometrial receptivity, resulting in down regulation of its receptors and making endometrial cells unresponsive to secreted hCG by hatched blastocyst [10].

Implications for research

It is still possible considering the mechanism of action that a specific cohort of patients may benefit, when given a specific dose of IU hCG, at a specific time prior to transfer. It is feasible that a certain combination of variables will lead to IU hCG intervention causing significant change in pregnancy outcomes following IVF-ET; one must consider the possibility that this change may be either beneficial or detrimental to implantation success rates. It is important to bear in mind that given there is no significant evidence in favour of the intervention, it is also possible that in specific cohorts this intervention may negatively impact outcomes. Potentially, those experiencing a benefit with the intervention are cancelling out those negatively impacted, and thus neither significant outcomes are able to be identified in the analysis. However, the RCTs conducted thus far do not cumulate to a large enough number for subgroup analysis. The subgroup analysis conducted, of timing of hCG and type of cycle (fresh v frozen), did not reveal any significant outcomes. However, it may be that a cohort with a specific combination of these variables will have a significant response to this intervention, further research is needed to define this cohort.

Conclusions

This meta-analysis is important in providing an updated assessment of the impact of IU hCG instillation on IVF-ET at the blastocyst stage. We found that combining the available data, there is in fact no significant impact on ART outcomes comparing the IU hCG groups to controls. Further studies are required to determine if cohorts with a specific combination of features: type of infertility, dose of IU hCG, timing of hCG instillation and type of cycle; have a significant outcome with this intervention.

Study funding/competing interest(s):

This study did not receive any funding. The authors have no financial, personal, intellectual and professional conflicts of interest to declare. A protocol was not created for this study, and it was not registered.

Contribution to authorship

S.M. wrote the manuscript; S.M. and E.T. were involved in the study design; S.M., E.T., L.S.K. and B.P.J. were involved in the literature search, analysis of the studies and drafting of the manuscript; S.M., E.T, L.S.K. and B.P.J. were involved in data collection; S.S. and J.B.N. were responsible for supervision and mentorship. E.T. and S.M. were responsible for statistical analysis. E.T. and S.S were responsible for study overview and drafting and revision of the manuscript. E.T. generated the topic of the manuscript. J.B.N. is the senior author of the manuscript and takes responsibility for its content. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy and integrity of any portion of the work are appropriately investigated and resolved.

Funding statement

No funding.

Declaration of Competing Interest

The authors declare there is no conflict of interests.

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