Lasers Med Sci (2018) 33:655–666
https://doi.org/10.1007/s10103-017-2372-x

REVIEW ARTICLE

The effect of laser-assisted hatching on pregnancy outcomes of cryopreserved-thawed embryo transfer: a meta-analysis of randomized controlled trials

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Received: 13 May 2017 / Accepted: 23 October 2017 / Published online: 7 November 2017

Abstract It is well known that laser-assisted hatching (LAH) is the most popular and ideal embryo hatching technology, but the relevance to pregnancy outcomes of cryopreserved-thawed embryo transfer (ET) is controversial. The purpose of this meta-analysis was to evaluate the effects of LAH on pregnancy outcomes of cryopreserved-thawed ET. We searched for relevant studies published in the PubMed, EMBASE, and Cochrane Central databases up to March 2017. This meta-analysis was primarily used to evaluate the effect of laser-assisted hatching on assisted reproductive outcomes: clinical pregnancy, embryo implantation, multiple pregnancy, miscarriage, and live birth. Using the Mantel-Haenszel fixed effects model and random effects model, we determined the summary odds ratios (OR) with 95% confidence intervals (CIs). There were 12 randomized controlled trials (more than 2574 participants) included in our analysis. The rates of clinical pregnancy (OR = 1.65, 95% CI = 1.24–2.19, I^2 = 49), implantation (OR = 1.59, 95% CI = 1.06–2.38, I^2 = 82%), multiple pregnancy (OR = 2.30, 95% CI = 1.30–4.07, I^2 = 33%), miscarriage (OR = 0.86, 95% CI = 0.50–1.48, I^2 = 0%), and live birth (OR = 1.09, 95% CI = 0.77–1.54, I^2 = 0%) revealed comparable results for both groups. In summary, this meta-analysis demonstrates that LAH is related to a higher clinical pregnancy rate, embryo implantation rate, and multiple pregnancy rate in women with cryopreserved-thawed embryos. However, LAH is unlikely to increase live birth rates and miscarriage rates. Due to the small sample evaluated in the pool of included studies, large-scale, prospective, randomized, controlled trials are required to determine if these small effects are clinically relevant.

Keywords Laser-assisted hatching · Cryopreservation · Zona pellucida · Embryo transfer

Introduction

A successful cryopreservation program of supernumerary frozen embryos may undoubtedly increase the cumulative conception rates following treatment with assisted reproductive technology (ART), while also reducing pregnancy and implantation rates compared to fresh embryo transfer [1, 2]. It is known that cryopreservation procedures, including immersion in a cryoprotectant and temperature changes during the process of cryopreservation, may affect the physicochemical characteristics by making the zona pellucida (ZP) thicker and/or harder, resulting in embryonic hatching difficulties, and ultimately affect the natural hatching process of the blastocyst [1].

The ZP is a glycoprotein layer that surrounds the outside of the human embryos. It triggers the acrosome reaction and, in the frame of a physiological process after fertilization, undergoes biochemical modifications called zona hardening, which prevents polyspermy [3, 4]. The rupture of the ZP in the blastocyst stage is called the hatching process, and this event allows embryo implantation; failure at this stage can hinder embryo implantation. Artificial rupture of the ZP is known as assisted hatching (AH). This technique has been used since the late 1980s in an attempt to enhance the opportunities for implantation and clinical pregnancy during ART [3, 5].

MeiFang Zeng and SuQin Su contributed equally to this work.

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Table 1 Characteristics of the included studies

Study	Method for allocation	Allocation concealed	Blinding	Informed consent	Inclusion criteria	Exclusion criteria	Time of AH	Participants LAH/ control	Age
(De Croo, et al. 2013)	Computer-generated list	Unclear	Yes	Yes	First frozen-thawed embryo transfer after a fresh embryo transfer	Previous assisted hatching cycle	Day 3 embryos	146/148	Not stated
(Hebisha, et al. 2015)	Not stated	Unclear	Yes	Yes	Frozen(vitrified)-thawed embryo transfer	Not frozen(vitrified)-thawed embryo transfer	Day 3 embryos	90/90	Not stated
(Ge, et al. 2008)	Not stated	Unclear	Yes	Yes	Normal baseline FSH concentration (3–12 IU/l) fewer than five failed cycles including fresh IVF/ICSI embryo transfer cycles and/or frozen-thawed embryo transfer cycles	uterine abnormality or low fertilization capacity (rate of fertilization <20% and late ICSI following fertilization failure of IVF)	Day 3 embryos	100/100	31.1 + 4.7/30.4 + 4.2
(Petersen, et al. 2006)	Randomized table	Unclear	Yes	Yes	Cryopreserved-thawed (CT) embryos	Not stated	Day 3 embryos	110/110	32.5 ± 4.0/30.5 ± 4.4
(Ng, et al. 2005)	Not stated	Unclear	Yes	Yes	≥ 2 frozen embryos available for transfer	> 3 stimulated IVF cycles; only one frozen embryo available for transfer; frozen embryos replaced in stimulated IVF cycles	Day 3 embryos	80/80	35.0/35.0
(Balaban, et al. 2006)	Not stated	Unclear	Yes	Yes	Male or unexplained fertility submitted to ICSI in the last 24 months with frozen/ thawed embryos	Not stated	Day 3 embryos	183/183	32.4 + 3.3/32.7 + 3.1
(Debrock, et al. 2011)	Sealed envelopes	Unclear	Yes	Yes	Frozen/vitrified	Biopsied in the context of PGD, embryo reception cycles, cycles with thawed/warmed embryos from unknown frozen origin (after transport from other center) and cycles with a higher number of embryos transferred than the number of embryos on which mQLAZT was performed	Day 3 embryos	302/317	32.27 + 4.42 (22.2–43.6) 32.74 + 4.34 (22.5–43.2)
(Elhelw, et al. 2005)	Not stated	Unclear	Unclear	Yes	Frozen-thawed embryo transfer	Not stated	Not stated	37/37	Not stated
(Kanyo, et al. 2016)	Registration number	Unclear	Yes	Yes		Not stated	Day 3 embryos	203/210	33.35 ± 3.3/33.04 ± 3.1

Table 1 (continued)

Study	Method for allocation	Allocation concealed	Blinding	Informed consent	Inclusion criteria	Exclusion criteria	Time of AH	Participants LAH/ control	Age
					Thaw–transfer cycles within a 12-month				
(Nagy, et al. 1999)	Not stated	Unclear	Unclear	Yes	Frozen-thawed embryo transfer	Not stated	Not stated	20/18	Not stated
(Valojerdi, et al. 2008)	Not stated	Unclear	Unclear	Yes	Frozen-thawed embryos	Not stated	Day 3 embryos	90/90	30.86 + 5.82/29.85 + 5.14
(Lu, et al. 2016)	Registration number	Unclear	Yes	Yes	Previous repeated failure	Not stated	Day 3 embryos	61/61	32.2 ± 3.6/31.5 ± 3.1

Artificially interfering with the ZP might improve implantation. A variety of techniques have been used to assist with embryo hatching, including partial zona dissection, zona drilling, and zona thinning, making use of acidic Tyrode's solution, proteinases, piezo vibrator manipulators, and lasers [6]. The use of laser-assisted hatching (LAH) first appeared in the scientific literature since the early 1990s [7–9]. In a large meta-analysis, Martins et al. demonstrated that LAH is one of the best, safest, and most effective AH methods at present [10]. In recent years, use of the non-contact infrared diode lasers has become more frequent compared with previous AH methodologies, since it allows rapid, controlled, and safe microdissection of the ZP [11]. The use of a laser for AH was initially developed to provide a precise and controlled method that could also be standardized between patients and operators [8]. Numerous studies have assessed the effects of lasers on embryos from both human and animal models, and have demonstrated no negative impacts on embryo development in vitro [12]. Furthermore, a few clinical studies have provided data following the use of LAH in human infertility patients. Zona thinning using laser is associated with a higher hatching rate than creating a perforation in the zona [13]. Little information exists in the literature with respect to the role of LAH in cryopreserved-thawed ET cycles. Compared with other methods, laser AH is the most popular and ideal technology. However, the clinical relevance of laser-assisted hatching remains controversial and elusive in cryopreserved-thawed embryo transfer.

The possible mechanisms by which LAH could ameliorate embryo implantation is that ZP hardening caused by in vitro fertilization/intracytoplasmic sperm injection(IVF/ICSI) and cryopreservation might make hatching difficult, which could be resolved by LAH. The clinical relevance of LAH remains controversial and elusive in cryopreserved-thawed ET. Moreover, no study has included a sufficient sample to effectively assess the influence of LAH on assisted reproduction outcomes. Here, a meta-analysis was performed to determine whether pregnancy rates can be improved if the zona barrier is compromised by LAH during cryopreserved-thawed ET.

Materials and methods

Search strategy

A literature search of the PubMed, EMBASE, and Cochrane Central databases was performed by employing the medical keywords "laser-assisted hatching" or "laser- assisted hatching". Studies were obtained from electronic databases and by scanning the reference lists of papers by two independent reviewers. The search was performed up to March, 2017.There was no restriction on language and publication year.

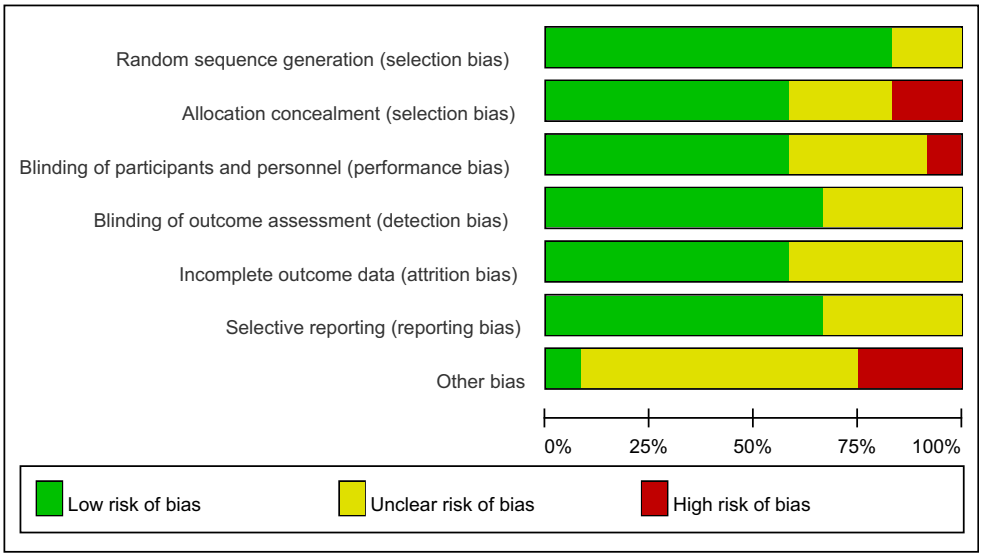

Fig. 1 Methodological quality summary: review authors' judgements about each methodological quality item for each included study

Inclusion and exclusion criteria

Only reports meeting the following inclusion criteria were included in the meta-analysis. Eligibility inclusion criteria were (1) randomized controlled trials, (2) cryopreserved-thawed ET, (3) human LAH embryos compared with a control group in which embryos were not submitted to LAH, (4) study should provide comparative data on clinical outcome after ET, and (5) human ET following IVF or ICSI, or both. Exclusion criteria were (1) non-randomized studies (for example, studies with evidence of inadequate sequence generation such as alternate days, patient numbers), (2) animal embryos or fresh embryos, (3) mechanical and chemical AH methodologies, (4) trials directly comparing different AH methods (without a no hatching control group), and (5) preimplantation genetic diagnosis (PGD).

Data extraction and quality assessment

Two review authors (MF Zeng and SQ Su) independently extracted data from eligible studies which was used to collect raw data. Any disagreement was resolved by discussion. Study characteristics and outcome data were generated from 12 eligible studies (Table 1). The Cochrane risk of bias tool was used for the risk assessment [14] (Figs. 1 and 2). The criteria consist of seven items related to selection bias (random sequence generation and allocation concealment), performance bias (blinding of participants and personnel), detection bias (blinding of outcome assessment), attrition bias (incomplete outcome data), reporting bias (selective outcome reporting), and other sources of bias.

Outcome measures

Our primary outcome measures chosen for the meta-analysis were the clinical pregnancy rate per randomized couple and implantation rate per embryo transfer. The secondary outcome measures included multiple pregnancy rate per clinical pregnancy, live birth rate, and miscarriage rate.

Statistical analysis

All statistical analyses were carried out with Rev. Man software [version 5.3]. For the included studies, the dichotomous data results for each of the studies eligible for meta-analysis were expressed as an odds ratio (OR) with 95% confidence intervals (CI). These results were combined for the meta-analysis using the Mantel-Haenszel model when using the random effects model and fixed effects model.

The number of women who were randomly allocated was considered as the total number of participants. Statistical heterogeneity between studies was evaluated by the chi-squared test and the I^2 statistic. An I^2 value greater than 50% is considered to represent a substantial heterogeneity. To evaluate the power, $p < 0.05$ was considered statistically significant.

Results

A total of 453 available publications were identified after the primary comprehensive literature research using aforementioned strategy: 131 from PubMed, 247 from EMBASE, and 75 from the Cochrane Library database (Fig. 11). The titles and abstracts of identified records were examined to exclude irrelevant papers, resulting in 113 potentially eligible studies. Twenty-seven of the 39 articles did not meet the study inclusion and were excluded. After application of our inclusion and exclusion criteria, 12 randomized controlled trials (RCTs) with a total of 2574 participants were identified. The main characteristics and quality features of the 12 included trials are presented in Table 1. All of the 12 studies were

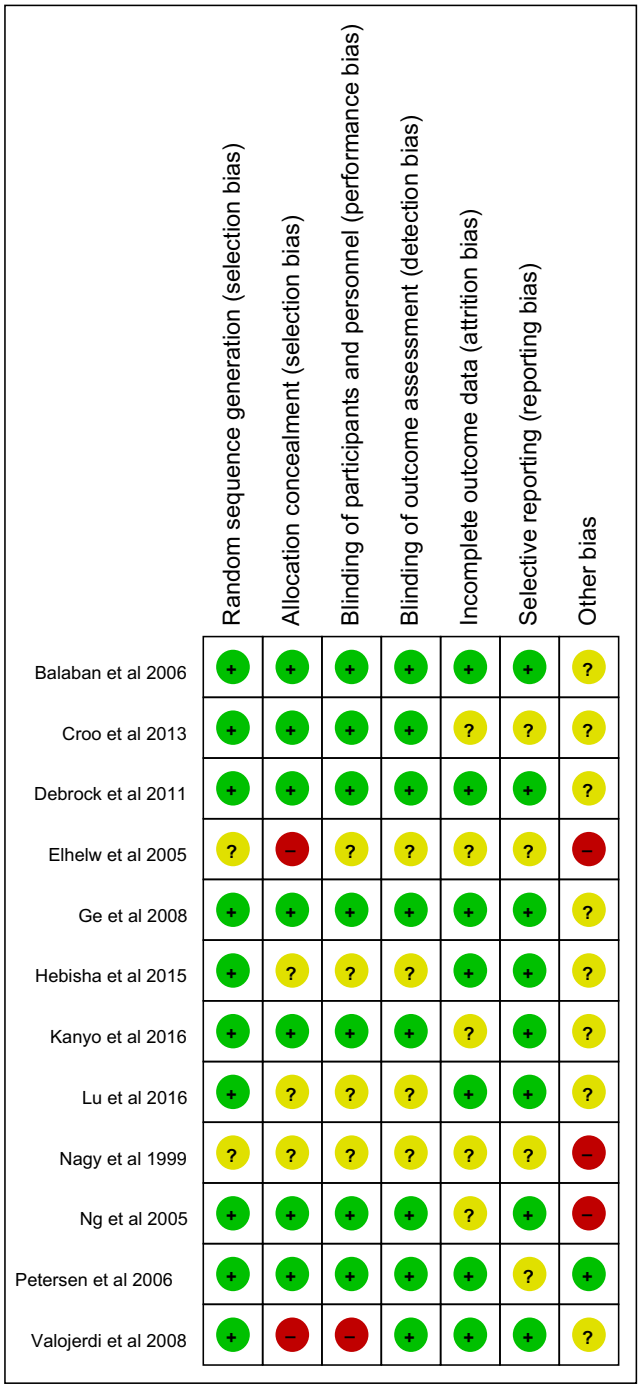

Fig. 2 Methodological quality summary: review authors' judgements about each methodological quality item for each included study

randomized studies and were considered to be of adequate quality for the meta-analysis.

Clinical pregnancy rate per couple Eleven RCTs investigated the influence of LAH on pregnancy rate per couple of cryopreserved-thawed ET (Fig. 3). A significant difference was detected between the two groups for clinical pregnancy rate per couple (OR = 1.65, 95% CI = 1.24–2.19). No heterogeneity was detected and the I^2 was 49%.

Implantation rate per embryo transfer There were nine RCTs that reported on the implantation rate (Figs. 4, 5, 6, 7). A significant difference in implantation rate was observed in the meta-analysis (OR = 1.59; 95% CI = 1.06–2.38). High heterogeneity was observed (I^2 = 82%). Four trials reported statistically significant benefits of LAH; three reported non-significant trends in favor of LAH and one reported a trend toward a lower implantation rate following LAH. However, the embryo implantation rate of this article showed substantial heterogeneity. Although numerous subgroup analyses were carried out, not all of them revealed statistically significant results.

Live births per couple Four RCTs investigated the effect of LAH on live births (Fig. 8). Evidence of a significant difference was not detected between the two groups for the live birth rate per couple favoring LAH (OR = 1.09, 95% CI = 0.77–1.54). No heterogeneity was detected and the I^2 was 0%.

Multiple pregnancy Five RCTs investigated the effect of LAH on multiple pregnancies (Fig. 9). Compared with women in the control group, women who underwent LAH had a significant increase in multiple pregnancies (OR = 2.30, 95% CI = 1.30–4.07), without heterogeneity (I^2 = 33%).

Miscarriage rate Five RCTs investigated the effect of LAH on the miscarriage rate (Fig. 10). No evidence of a significant difference was detected between the two groups for the miscarriage rate (OR = 0.86, 95% CI = 0.50–1.48). No heterogeneity was detected and the I^2 was 0%.

Discussion

This comprehensive meta-analysis of 12 RCTs analyzed the effects of LAH on pregnancy outcomes of cryopreserved-thawed ET in more than 2574 participants. However, two of the 12 articles provided only data [15, 16]. Eleven RCTs investigated the influence of LAH on the pregnancy rate per couple undergoing cryopreserved-thawed ET. There was evidence of a significant difference in the clinical pregnancy rate per couple, favoring the LAH group. There was evidence of a small but significant difference in the implantation rate per ET and no difference in the miscarriage rate. Overall, there was no difference in the live birth rate between the LAH group and the control group in cryopreservation ET. Evidence of a difference was found between the LAH group and the control group for the multiple pregnancy rate. We grouped these comparisons into those involving reproduction outcomes with

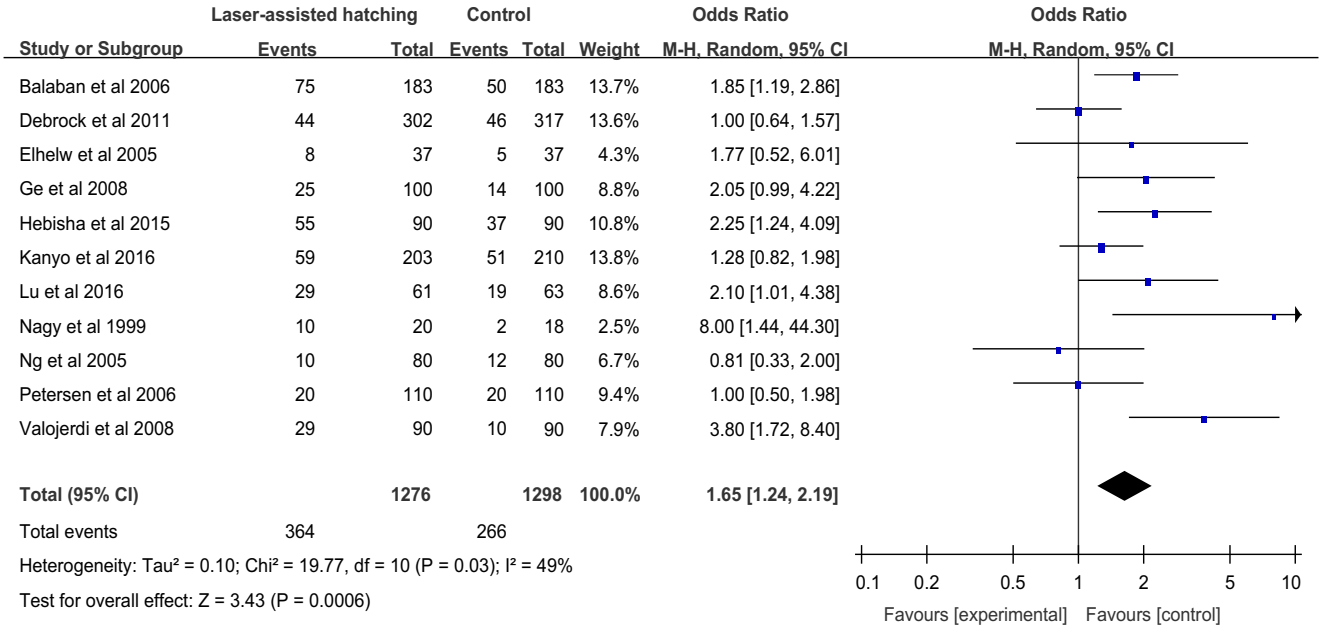

Fig. 3 Forest plot of comparison: clinical pregnancy rate per couple

clinical pregnancy, embryo implantation, multiple pregnancy, miscarriage, and live birth.

In this meta-analysis, the primary outcomes were the clinical pregnancy rate and the implantation rate. Our meta-analysis included 11 RCTs that reported clinical pregnancy rates; there were 630 pregnancies in 2574 patients, 364 in the LAH group, and 266 in the control group. LAH tended to increase the clinical pregnancy rate when taking into account pooled data from 11 articles (OR = 1.65, 95% CI = 1.24–2.19). Indeed, this study observed statistically significant differences in the clinical pregnancy rates after LAH, suggesting that LAH contributes to favorable clinical outcomes in cryopreserved-thawed ET. No significant heterogeneity was observed in our meta-analysis. LAH involves completely removing the zona pellucida and led to a significant increase in clinical pregnancy rates. LAH involves the controlled and rapid microdissection of the ZP, and its safety has been shown in both animals and humans. Laser irradiation induces photolytic ablation of the zona pellucida without changing the cytoplasmic structure [1].

One of the most important reasons for the low rate of embryo implantation is the failure of hatching in IVF/ICSI [17].The comparable embryo implantation rates per ET between the two groups indicate that embryo implantation was

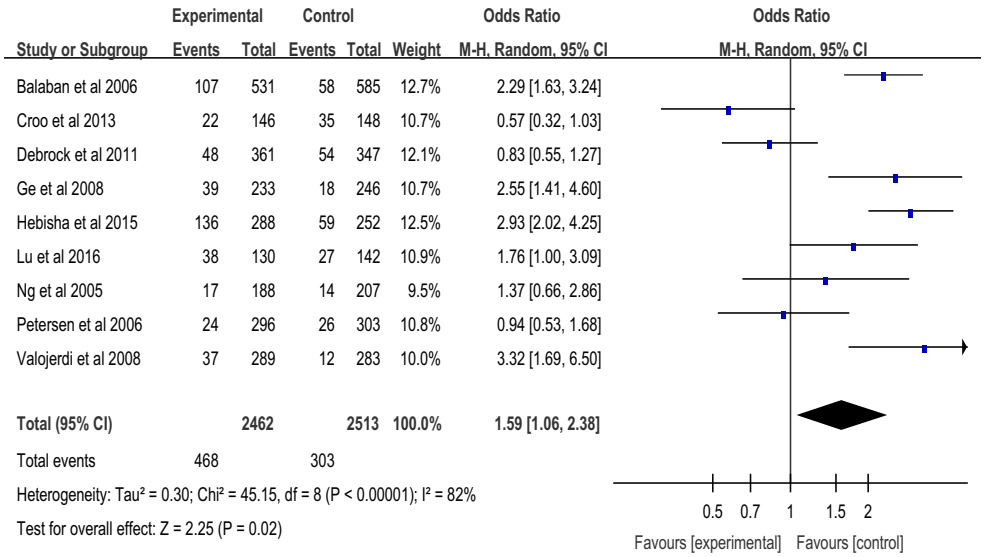

Fig. 4 Forest plot of comparison: Implantation rate per embryo transfer

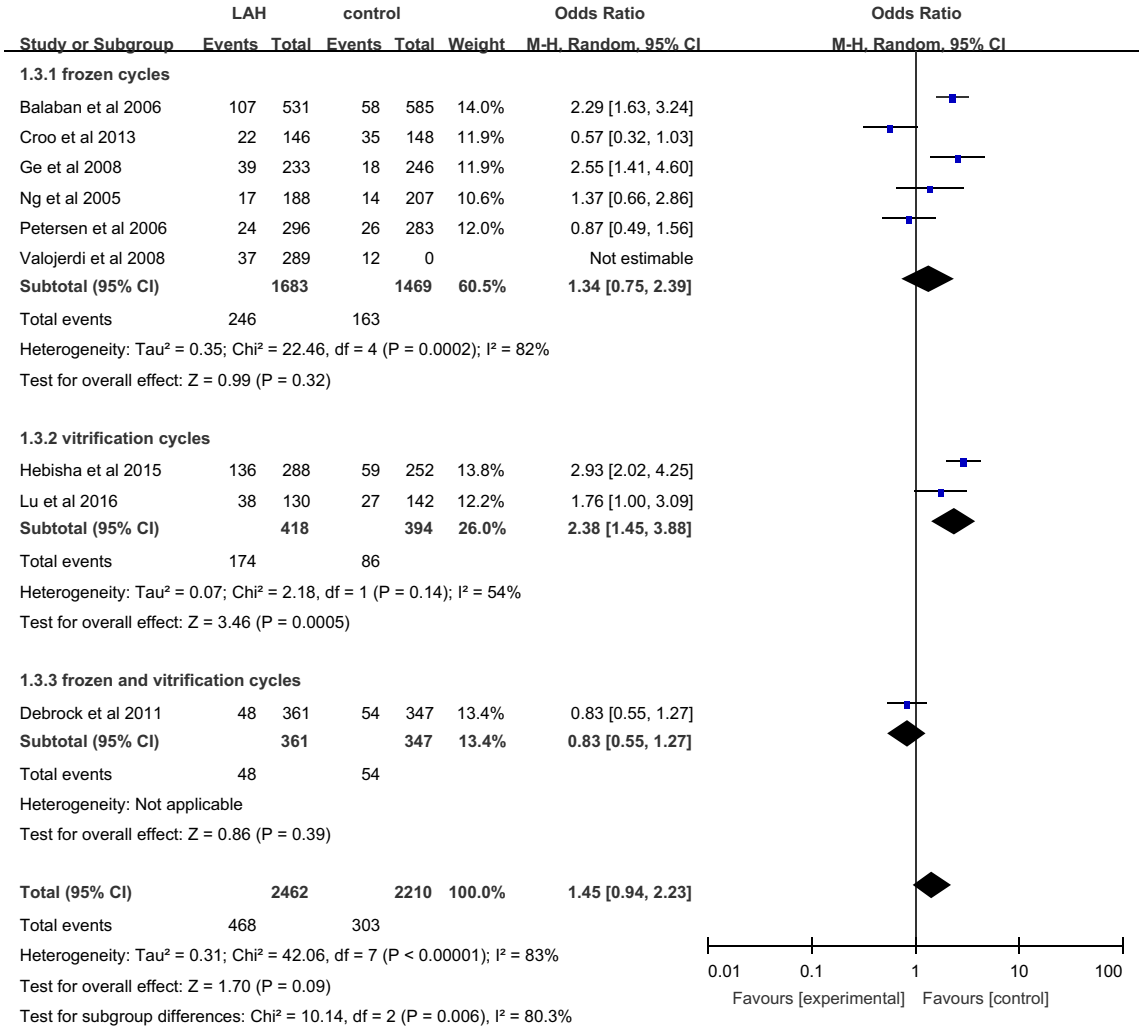

Fig. 5 Forest plot of comparison: Implantation rate per embryo transfer grouped by cryopreservation program

markedly enhanced after LAH (19% for LAH versus 12% for the control group) before the transfer of cryopreserved-thawed embryos. Elasticity and thinning of the ZP are important in the hatching process, which can be unfavorably affected by the cryopreservation-thawing process [18–20]. The end result will be failure of the embryonic ZP to rupture following blastocyst expansion, which is a prerequisite for embryo implantation [21]. There are three possible mechanisms for improving embryo implantation in the process of AH [17]. First, secondary medium conditions or cryopreservation procedures might make the embryonic ZP harden and/or thicken [22, 23], which might lead to hatching difficulty. By thinning, drilling or other methods, AH could mechanically promote the hatching process. Second, studies in human and animal models found that AH resulted in earlier hatching than in non-assisted hatching embryos [24, 25]. Facilitation of earlier embryonic hatching might be particularly important given that the short window of endometrial receptivity appeared to be shifted 1–2 days earlier in cycles with ovarian stimulation for assisted reproduction treatment compared with natural cycles [26, 27]. Third, the artificial gap produced by hatching may also serve as a channel for the exchange of metabolites, growth factors, and messages between the embryo and the endometrium [18]. Our meta-analysis shows the beneficial effect of laser ZP thinning when compared with the control group in terms of implantation rates. However, it is difficult to assess the value of LAH in cryocycles due to considerable heterogeneity (I^2 = 82%) regarding the cryopreservation program, conception mode and extent of LAH, clinical and demographic patient characteristics, sample size, and study design. Firstly, regarding the extent of LAH, in our meta-analysis, all RCTs used laser technology, and the ZP was completely opened [28] or thinned [1, 17, 21, 29–32]; this was not reported in one study [33]. The exact mechanism and value LAH have never been described in animal models, and potential harmful effects on embryo development cannot be excluded. It appears that the extent of ZP thinning area by LAH could affect the rates of implantation for cryopreserved-thawed ETs at the cleavage stage [34,

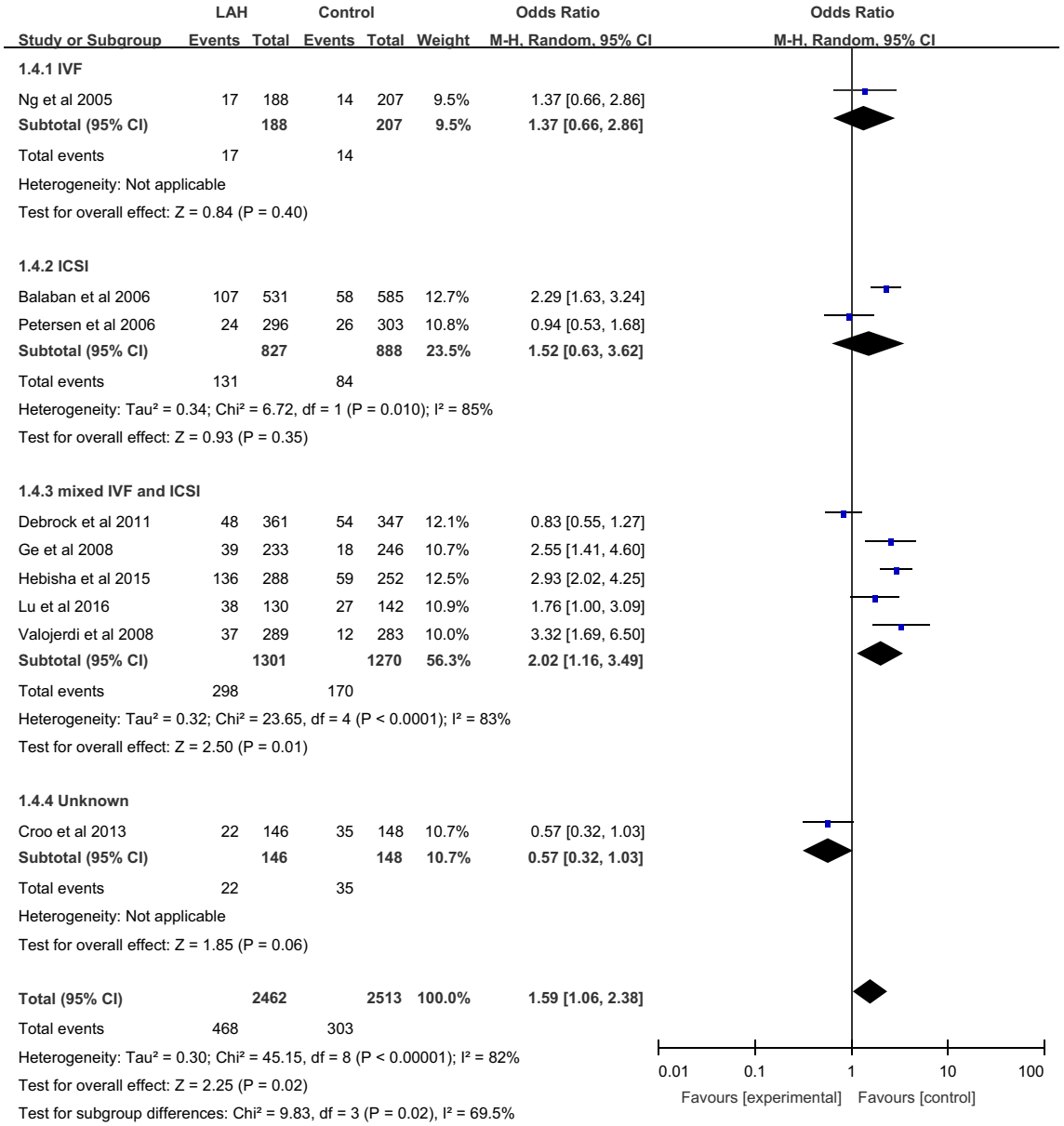

Fig. 6 Forest plot of comparison: Implantation rate per embryo transfer grouped by conception mode

35]. In our subgroup analysis, the extent of LAH was not found to affect the heterogeneity of the implantation rate. Secondly, the method of cryopreservation may influence clinical outcomes. Hiraoka et al. showed that vitrification can increase the hardness of the ZP and affect hatching compared with programmed freezing [4, 34]. In our meta-analysis, some studies used a slow freezing method for cryopreservation [1, 17, 21, 28, 33]; some studies used the vitrification method [31, 32] and others used embryos that were cryopreserved with both the slow freezing method and the vitrification method [30]. In our subgroup analysis of the method of cryopreservation, this did not affect the heterogeneity in the implantation rate. Finally, the conception mode may influence the implantation rate. In some studies, all couples had been subjected to ICSI for male factor or unexplained infertility [1, 29]; in some studies, all couples had been subjected to the IVF/ET program [21]; in other studies, all couples used both mixed IVF and ICSI [17, 28, 30–32]. Nevertheless, in our subgroup analysis of conception mode, the implantation rate was not the origin of heterogeneity. Moreover, the studies did not adjust for the same confounders. Although most of the potential factors that interfere with embryo implantation were eliminated, the embryo implantation mechanism is extremely complex. Further well-designed studies considering more covariates are required to examine the differences between the LAH group and the control group.

In this meta-analysis, only four of the 12 studies reported on the live birth rate. There is no evidence that LAH has an

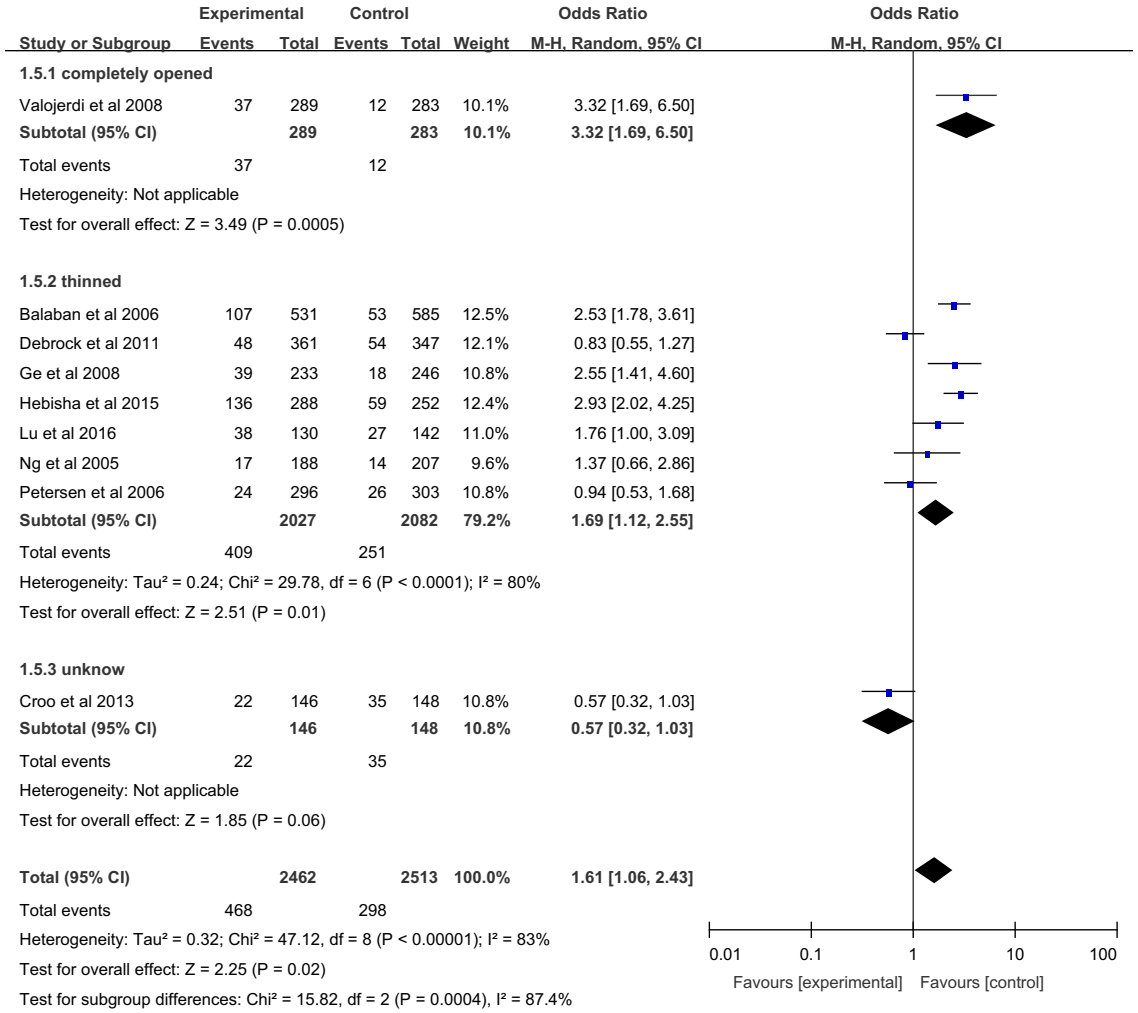

Fig. 7 Forest plot of comparison: Implantation rate per embryo transfer grouped by extent of LAH

influence on the live birth rate as yet. It was disappointing that the conclusions of the meta-analysis were restricted by the scarcity of existing data. That only four of the comprised studies reported live birth data show haste on the part of some authors to disseminate data limited to short-term outcomes; these data are insufficient.

In general, there was a significant increase in multiple pregnancies per clinical pregnancy, indicating that LAH increases the chance of multiple pregnancy. One of the serious complications of assisted reproductive technology (ART) is multiple pregnancy, especially triplets or more [36]. The reason for the increase in multiple pregnancies can be ascribed to an increase

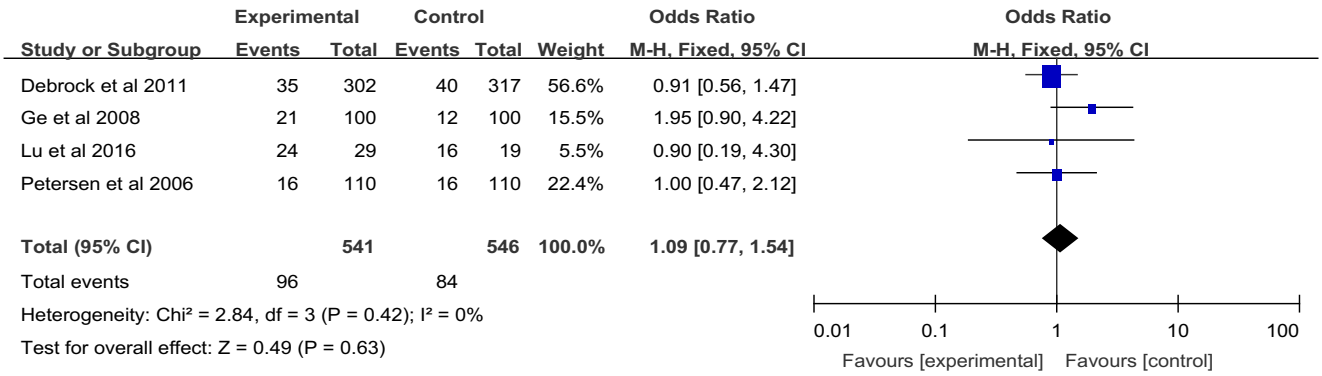

Fig. 8 Forest plot of comparison: Live birth per couple

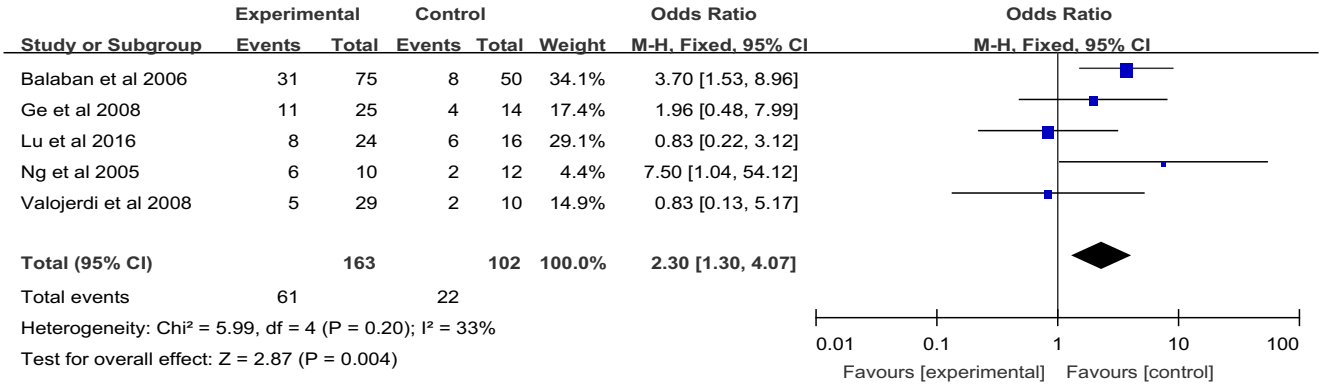

Fig. 9 Forest plot of comparison: multiple pregnancy rate

in the implantation rate, leading to higher pregnancy rates or monozygotic twinning, or both, with LAH. Reducing the number of embryos transferred to eliminate monozygotic twinning must be taken into consideration. In the clinic, embryo implantation is expressed per embryo transferred, without regard to the number of patients. However, each woman is normally transferred with more than one embryo, leading to an embryo clustering effect. Without a doubt, blastocyst transfer is a way to reduce the burden of multiple pregnancies on children, owing to the fact that a lower number of embryos is transferred. A statistically valid and easier approach is to express implantation is randomized per woman [37]. This approach requires, however, that the number of women with at least one gestation sac is reported, which is not the case in practice.

This meta-analysis did not find sufficient proof to reach a conclusion on the influence of LAH on miscarriage rates. Miscarriage rate is a key element when assessing a new mode of treatment, and it has a significant influence on treatment efficiency and live birth rates. The first trimester of pregnancy refers to the first 14 weeks of the pregnancy cycle. Most spontaneous miscarriages occur during the first trimester after ART, which has a direct connection with the potential of embryonic development. In our meta-analysis, in the LAH group, the first trimester miscarriage rate was not higher compared with the control group. LAH was initially developed to offer a precise and controlled means that could also be standardized between patients and manipulators [8]. Furthermore, LAH is a no-touch technique and is known to produce no thermal or mutagenic side effects [8, 38]. LAH is a rapid technique that can be completed in minutes. Because of the small sample assessed by the pool of included articles, no appropriate conclusions could be drawn with regard to miscarriage or live birth rates.

The strength of the present meta-analysis lies in its RCT studies and no significant evidence of publication bias. Despite its strengths, there are some weaknesses in our meta-analysis. The quality of evidence of the included studies is poor. Selection bias is an inevitable problem, but it is difficult to overcome this problem through statistical methods. Due to the low quality of available data, there is still no definitive conclusion regarding the live birth or miscarriage rates. A large RCT is needed in the future. There was significant heterogeneity in the meta-analysis of implantation rate (I^2 = 82%), making the results unstable and less convincing, suggesting some effect of between-studies variation on the results. Thus, we ran a subgroup analysis to examine the source of heterogeneity. However, these variables could not

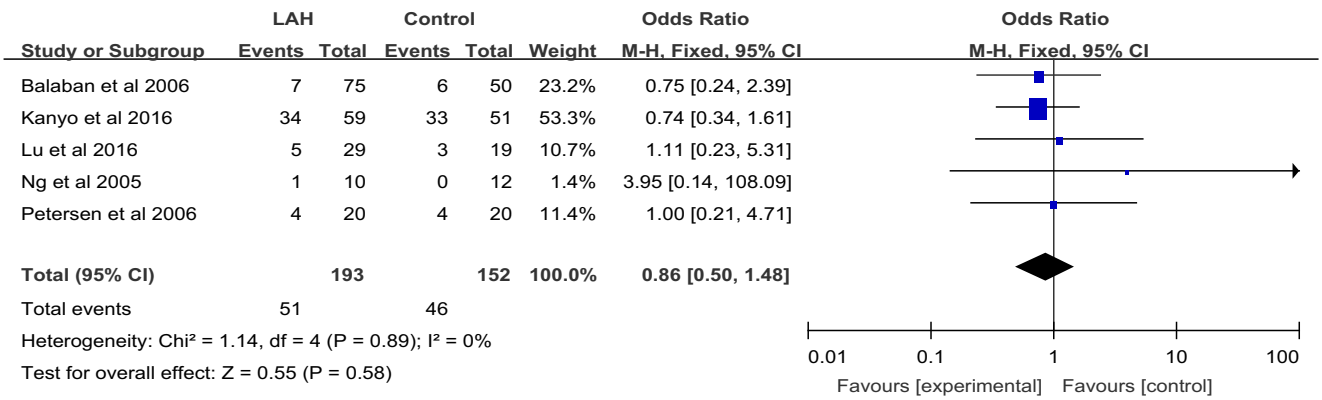

Fig. 10 Forest plot of comparison: miscarriage rate

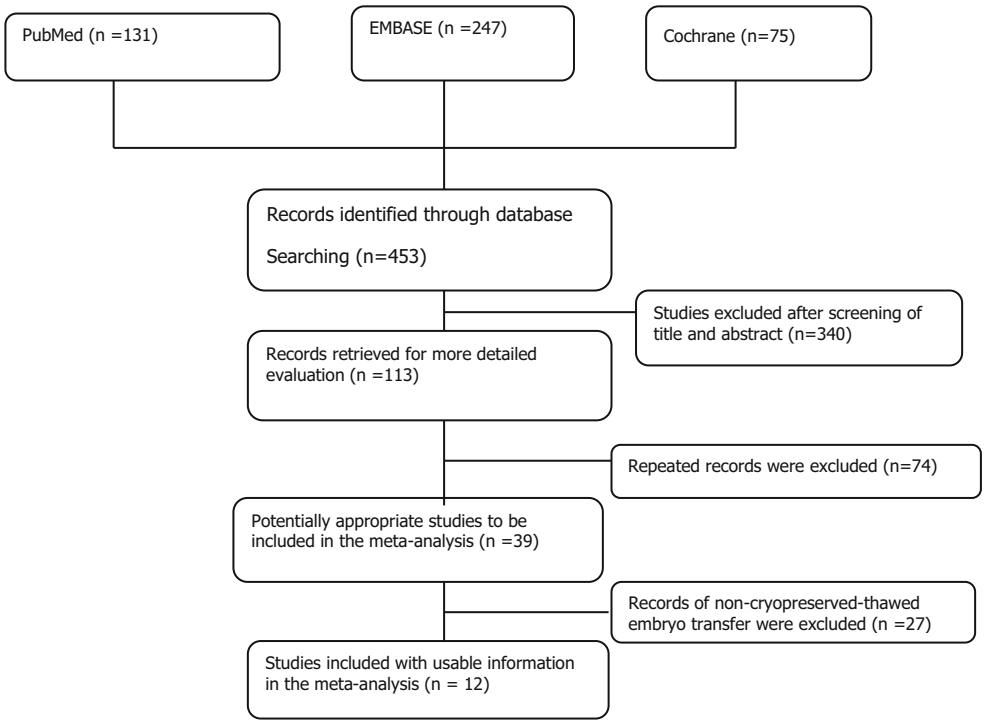

Fig. 11 Published related comparative studies in the PubMed, EMBASE, and Cochrane Library databases

thoroughly settle the origin of heterogeneity, recommending that other unknown confounding variables might be the origin of heterogeneity. Moreover, the studies did not adjust for the same confounders. Further, well-designed studies considering more covariates are needed to investigate the association between LAH and the implantation rate.

In conclusion, on the basis of evidence provided by the available randomized studies, LAH seems to be an efficient method to improve the clinical pregnancy rate and implantation rate. The routine use of LAH on all embryos in IVF/ICSI patients is neither scientific nor appropriate. Selective application of LAH may enhance hatching ability in ART.

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