Factors related to clinical pregnancy after vitrified–warmed embryo transfer: a retrospective and multivariate logistic regression analysis of 2313 transfer cycles

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STUDY QUESTION: What factors does multivariate logistic regression show to be significantly associated with the likelihood of clinical pregnancy in vitrified–warmed embryo transfer (VET) cycles?

SUMMARY ANSWER: Assisted hatching (AH) and if the reason to freeze embryos was to avoid the risk of ovarian hyperstimulation syndrome (OHSS) were significantly positively associated with a greater likelihood of clinical pregnancy.

WHAT IS KNOWN ALREADY: Single factor analysis has shown AH, number of embryos transferred and the reason of freezing for OHSS to be positively and damaged blastomere to be negatively significantly associated with the chance of clinical pregnancy after VET. It remains unclear what factors would be significant after multivariate analysis.

STUDY DESIGN, SIZE, DURATION: The study was a retrospective analysis of 2313 VET cycles from 1481 patients performed between January 2008 and April 2012. A multivariate logistic regression analysis was performed to identify the factors to affect clinical pregnancy outcome of VET.

PARTICIPANTS/MATERIALS, SETTING, METHODS: There were 22 candidate variables selected based on clinical experiences and the literature. With the thresholds of \( \alpha_{entry} = \alpha_{removal} = 0.05 \) for both variable entry and variable removal, eight variables were chosen to contribute the multivariable model by the bootstrap stepwise variable selection algorithm (\( n = 1000 \)). Eight variables were age at controlled ovarian hyperstimulation (COH), reason for freezing, AH, endometrial thickness, damaged blastomere, number of embryos transferred, number of good-quality embryos, and blood presence on transfer catheter. A descriptive comparison of the relative importance was accomplished by the proportion of explained variation (PEV).

MAIN RESULTS AND THE ROLE OF CHANCE: Among the reasons for freezing, the OHSS group showed a higher OR than the surplus embryo group when compared with other reasons for VET groups (OHSS versus Other: OR: 2.145; CI: 1.4–3.286; Surplus embryos versus Other, OR: 1.152; CI: 0.761–1.743) and high PEV (marginal 2.77%, \( P = 0.2911 \); partial 1.68%; CI of area under receptor operator characteristic curve (ROC): 0.5576–0.6000). AH also showed a high OR (OR: 2.105, CI: 1.554–2.85) and high PEV (marginal 1.97%; partial 1.02%; CI of area under ROC: 0.5344–0.5647). The number of good-quality embryos showed the highest marginal PEV and partial PEV (marginal 3.91%, partial 2.28%; CI of area under ROC: 0.5886–0.6343).

LIMITATIONS, REASONS FOR CAUTION: This was a retrospective multivariate analysis of the data obtained in 5 years from a single IVF center. Repeated cycles in the same woman were treated as independent observations, which could introduce bias. Results are based on clinical pregnancy and not live births. Prospective analysis of a larger data set from a multicenter study based on live births is necessary to confirm the findings.
WIDER IMPLICATIONS OF THE FINDINGS: Paying attention to the quality of embryos, the number of good embryos, AH and the reasons for freezing that are associated with clinical pregnancy after VET will assist the improvement of success rates.

STUDY FUNDING/COMPETING INTEREST(S): This study was financially supported by the Scientific and Technological Research Projects of Shaanxi Province (project number: 2011k15-02-01). All the authors have no conflict of interest to declare.

TRIAL REGISTRATION NUMBER: N/A.

Key words: vitrified–warmed embryo transfer (VET) / clinical pregnancy / multivariate logistic regression analysis

Introduction

The increased adoption of the elective single embryo transfer (ET) policy (e-SET) has resulted in more surplus embryos for cryopreservation to maximize the cumulative effectiveness of an IVF cycle (Michellmann and Nayudu, 2006; McLernon et al., 2010). Because vitrification appears to be an efficient and safe method for preservation (Loutradi et al., 2008; Shi et al., 2012), the prevalence of vitrification increased in the UK and worldwide in the last decade (Brison et al., 2012). During the process of the vitrified–warmed embryo transfer (VET), there are some controversial factors, such as assisted hatching (AH), post-warm culture and the endometrial preparation protocol (Gelbaya et al., 2006; Fatemi et al., 2010; Debrock et al., 2011; Rato et al., 2012; Tomás et al., 2012).

Most previous studies have analyzed the association of these factors with the outcome of VET using one-factor analysis. Single-factor analysis has shown AH, number of embryos transferred and reason for freezing that are associated with clinical pregnancy after VET (Queenan et al., 1997; Van et al., 1997; El-Toukhy et al., 2003a,b; Berin et al., 2010; Kutlu et al., 2010). Few data that take account of confounding factors such as patient age, embryo quality, number of embryos to transfer, different laboratory procedure and clinical alternations. It is obvious that randomized controlled trials to take account of all the possible factors would be inpracticable. However, multivariable analysis of clinical practice might provide new insights into the importance of these factors. With this in mind, a multivariable logistic regression analysis of 2313 VET cycles was performed to determine which one or group of factors were significantly associated with the likelihood of clinical pregnancy of VET. The primary objective was to build a multivariable analysis model and identify the factors which affect the successful outcome of VET.

Materials and Methods

Patients and data collection

The Assisted Reproduction Center is a public clinic in Shaanxi Province, China. This study was a retrospective analysis of the data from our center and was approved by the Ethics Review Board of the Maternal & Child Health Care Hospital of Shaanxi Province. The data were analyzed from patients with completed VET cycles. The following cycles were excluded: (i) cycles where no vitrified embryos survived after warming, (ii) cycles with blastocyst transfer; (iii) cycles with incomplete follow-up; (iv) cycles where embryos were not preserved by vitrification and (v) cycles with oocyte donation transfer. Overall we analyzed complete data set of 2313 VET cycles in 1481 patients between January 2008 and February 2012.

Ovarian stimulation and VET policy

Most patients used the standard long and short protocols with GnRH agonist (GnRH-a, Decapeptyl Germany) and recombinant FSH (GONAL-f, Merck Serono Italy; Puregon, Organon Netherlands) for controlled ovarian hyperstimulation (COH). Other protocols with or without human menopausal gonadotrophin (hMG, Li Zhu, China) were also adopted in COH according to the patients’ response to stimulation. 10 000 units of human chorionic gonadotrophin (hCG) was administered when ≥3 follicles were >18 mm. Oocyte retrieval was performed 36 h later by transvaginal ultrasonography-guided aspiration. Fertilization was performed using either conventional IVF or ICSI whilst incubated in fertilization media (Vitrolife, Sweden).

After fresh ET, patients’ surplus embryos were vitrified on Day 3. The VET was carried out in those patients who failed to conceive from fresh ET. In patients who were at a risk of ovarian hyperstimulation syndrome (OHSS), or presented with an endometrial cavity fluid complication, hydrosalpinx, abnormal endometrium or acute marital problems, no embryos were transferred and all were vitrified on Day 3. The vitrified embryos were warmed and transferred usually after a few months following oocyte collection.

Embryo vitrification and warming procedures

The vitrification and warming procedure was done according to standard protocols, as previously described (Shi et al., 2012). Tools and solutions required for vitrification were obtained from Kitazato (Kitazato BioPharma Co, Japan). The thawed embryos were observed after warming and again before transfer to assess for morphological survival. Embryos with >50% intact blastomeres and no signs of fracture of the zona pellucida (ZP) were defined as surviving. The presence of damaged blastomeres was recorded at the same time.

AH procedures

We started AH in our center from Jun 2009, with a laser treatment (ZILOS-sk; Hamilton Thorne Instruments Biosciences, Beverly, MA01915, USA). It was gradually performed on all vitrified Day 3 embryos after warming. It was generally performed an hour before ET. Embryos were placed under mineral oil within a 50 μl micro-droplet of G2.5 (Vitrolife, Sweden) on the stage of the microscope. A portion of the ZP was positioned in the circle of the laser beam in such a way that the perivitelline space was widest at the region targeted. With a setting of 100% power and 500 μs plus duration, the ZP was thinned to more than two-thirds of its initial thickness and a distance of 30–40 μm. After AH, the embryos were washed for several times and kept in fresh EmbryoGlue (Vitrolife, Sweden) until the time of the ET.
<table>
<thead>
<tr>
<th>Variables</th>
<th>Pregnant (n = 1133)</th>
<th>Not pregnant (n = 1180)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at COH (y)</td>
<td>29.3 ± 3.86</td>
<td>30.1 ± 4.46</td>
<td>0.0000a</td>
</tr>
<tr>
<td>Age at ET (years)</td>
<td>29.5 ± 3.84</td>
<td>30.3 ± 4.45</td>
<td>0.0000a</td>
</tr>
<tr>
<td>BMI (kg m(^{-2}))</td>
<td>21.6 ± 2.82</td>
<td>21.6 ± 2.86</td>
<td>0.9823a</td>
</tr>
<tr>
<td>Antral follicle count</td>
<td>14.1 ± 5.77</td>
<td>12.6 ± 5.43</td>
<td>0.0000a</td>
</tr>
<tr>
<td>Basal serum FSH level (mIU/ml)</td>
<td>6.8 ± 3.41</td>
<td>7.1 ± 3.75</td>
<td>0.0138a</td>
</tr>
<tr>
<td>Basal serum E2 level (pg/ml)</td>
<td>46.7 ± 47.23</td>
<td>46.9 ± 45.88</td>
<td>0.8971a</td>
</tr>
<tr>
<td>Length of embryo cryopreservation (month)</td>
<td>6.2 ± 4.71</td>
<td>6.5 ± 4.46</td>
<td>0.1710a</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>9.6 ± 1.54</td>
<td>9.4 ± 1.51</td>
<td>0.0021a</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>2.4 ± 0.54</td>
<td>2.3 ± 0.62</td>
<td>0.0323a</td>
</tr>
<tr>
<td>No. of embryos transferred (cycles, %)</td>
<td></td>
<td></td>
<td>0.1386b</td>
</tr>
<tr>
<td>1</td>
<td>38 (26.8)</td>
<td>104 (73.2)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>663 (51.6)</td>
<td>623 (48.4)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>432 (48.8)</td>
<td>453 (51.2)</td>
<td></td>
</tr>
<tr>
<td>No. of good-quality embryos</td>
<td>1.0 ± 0.95</td>
<td>0.6 ± 0.87</td>
<td>0.0000b</td>
</tr>
<tr>
<td>No. of good-quality embryos (cycle, %)</td>
<td></td>
<td></td>
<td>0.0000b</td>
</tr>
<tr>
<td>0</td>
<td>441 (38.9)</td>
<td>692 (61.1)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>333 (55.4)</td>
<td>268 (44.6)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>285 (62.1)</td>
<td>174 (37.9)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>74 (61.7)</td>
<td>46 (38.3)</td>
<td></td>
</tr>
<tr>
<td>Main etiology of infertility (cycles, %)</td>
<td></td>
<td></td>
<td>0.0614c</td>
</tr>
<tr>
<td>Tubal factor</td>
<td>656 (47.95)</td>
<td>712 (52.05)</td>
<td></td>
</tr>
<tr>
<td>Ovarian factor</td>
<td>94 (53.41)</td>
<td>82 (46.59)</td>
<td></td>
</tr>
<tr>
<td>Male factor</td>
<td>214 (53.77)</td>
<td>184 (46.23)</td>
<td></td>
</tr>
<tr>
<td>Other reasons</td>
<td>169 (45.55)</td>
<td>202 (54.45)</td>
<td></td>
</tr>
<tr>
<td>Type of infertility (%)</td>
<td></td>
<td></td>
<td>0.4130c</td>
</tr>
<tr>
<td>Primary infertility</td>
<td>649 (49.73)</td>
<td>656 (50.27)</td>
<td></td>
</tr>
<tr>
<td>Secondary infertility</td>
<td>484 (48.02)</td>
<td>524 (51.98)</td>
<td></td>
</tr>
<tr>
<td>COH protocol (cycles, %)</td>
<td></td>
<td></td>
<td>0.0000c</td>
</tr>
<tr>
<td>Short protocol</td>
<td>82 (33.7)</td>
<td>161 (66.3)</td>
<td></td>
</tr>
<tr>
<td>Long protocol</td>
<td>1013 (51.6)</td>
<td>950 (48.4)</td>
<td></td>
</tr>
<tr>
<td>Other protocol</td>
<td>38 (35.5)</td>
<td>69 (64.5)</td>
<td></td>
</tr>
<tr>
<td>Fertilization method (cycles, %)</td>
<td></td>
<td></td>
<td>0.2188c</td>
</tr>
<tr>
<td>ICSI</td>
<td>230 (46.6)</td>
<td>264 (53.4)</td>
<td></td>
</tr>
<tr>
<td>IVF</td>
<td>829 (49.2)</td>
<td>855 (50.8)</td>
<td></td>
</tr>
<tr>
<td>IVF + ICSI</td>
<td>74 (54.8)</td>
<td>61 (45.2)</td>
<td></td>
</tr>
<tr>
<td>The reason for freezing (cycles (%))</td>
<td></td>
<td></td>
<td>0.0000c</td>
</tr>
<tr>
<td>Surplus embryos after fresh ET</td>
<td>638 (43.4)</td>
<td>832 (56.6)</td>
<td></td>
</tr>
<tr>
<td>Ovarian hyperstimulation syndrome</td>
<td>439 (60.7)</td>
<td>284 (39.3)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>56 (46.7)</td>
<td>64 (53.3)</td>
<td></td>
</tr>
<tr>
<td>Post-thaw culture time (cycles, %)</td>
<td></td>
<td></td>
<td>0.0000b</td>
</tr>
<tr>
<td>2–4 h</td>
<td>62 (28.3)</td>
<td>157 (71.7)</td>
<td></td>
</tr>
<tr>
<td>18–20 h</td>
<td>1071 (51.2)</td>
<td>1023 (48.9)</td>
<td></td>
</tr>
<tr>
<td>AH (cycles, %)</td>
<td></td>
<td></td>
<td>0.0000c</td>
</tr>
<tr>
<td>No</td>
<td>110 (32.9)</td>
<td>224 (67.1)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1023 (51.7)</td>
<td>956 (48.3)</td>
<td></td>
</tr>
</tbody>
</table>

Continued
Endometrial preparation and post-thaw culture

Endometrial preparation was performed in spontaneous natural and artificial cycles. For the artificial cycles, exogenous estrogen and progestogen were administered to prime the endometrium, without the addition of a GnRH agonist. The endometrial preparation started with E2 2 mg three times a day, orally, from the start cycle. When the thickness of the endometrium was at least 7 mm, 60 mg/day of P (Progestosterone injection) was added 1 day before the transfer. In natural cycles, VET was planned 3 days after detection of the endogenous LH surge or administration of hCG. The embryos were warmed on the day of ET with a post-thaw culture time of 2–4 h before March 2009. After March 2009, the embryos were warmed in the afternoon on 1 day before ET with a post-warm-culture time of 18–20 h. Endometrial thickness was routinely measured on the day of transfer.

ET and pregnancy confirmation

The ET catheter (COOK IRELAND LTD, Ireland) was used for transfers. Before transfer, any vaginal and cervical secretions were gently removed from the vagina/cervix with small pledgets of cotton wool, moistened with warm normal saline. The mucus in the cervical canal was wiped away. After transfer, the catheter was checked for retained embryos and the presence of blood. After ET, all patients were given luteal support (Duphaston; progesterone injection). Clinical pregnancy was confirmed by the presence of a gestational sac.

Statistical analysis

There were 22 candidate variables identified from clinical experiences and the literature. Eleven variables were chosen by a bootstrapping stepwise variable selection algorithm \( (n = 1000) \) with the thresholds of \( \alpha_{\text{entry}} = \alpha_{\text{removal}} = 0.05 \) for both variable entry and variable removal. Three factors (age at ET, observed mitosis and observed compaction) were excluded and eight factors were chosen to contribute the multivariable logistic regression model. Age at COH and age at ET were two variables on behalf of the role of age on the results. Age at COH, indicating more messages of oocyte, was included instead of age at ET. For observed mitosis and observed compaction, the two variables were related to the time of observation, which differed much between post-thaw culture time (2–4 h and 18–20 h). To avoid interaction between these variables, the two variables (observed mitosis and observed compaction) were excluded. A receiver operating characteristic (ROC) curve was constructed to evaluate the performance of the multivariable model. A descriptive comparison of the relative importance was accomplished by the proportion of explained variation (PEV), a well-interpretable measure to quantify the importance of prognostic factors. The marginal PEV is a measure of the contribution of a given factor to the model univariately, whereas the partial PEV measures the decrease in explained variation when removing the prognostic factor from the model (Schumpe, 1993; Heinze and Schumpe, 2003). All analyses were performed with SAS statistical software (version 9.13).

Results

In 2313 cycles of VET, 1133 clinical pregnancies were obtained (49.0%). The patients and cycle characteristics by clinical pregnancy were summarized in Table I. A total of 22 candidate variables were tested by one-factor analysis. Fifteen factors were identified as being significantly different between two groups \( (P < 0.05) \): age at COH, age at ET, antral follicle count, basal serum FSH level, endometrial thickness, mean number of embryos transferred, mean number of good-quality embryos, protocol of COH, reason for freezing, post-thaw culture duration period, AH, observed mitosis, damaged blastomere, observed compaction, presence of blood on catheter.

Table I

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pregnant ( (n = 1133) )</th>
<th>Not pregnant ( (n = 1180) )</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial preparation protocol (cycles, %)</td>
<td></td>
<td></td>
<td>0.2279c</td>
</tr>
<tr>
<td>Natural cycle</td>
<td>364 (47.2)</td>
<td>407 (52.8)</td>
<td></td>
</tr>
<tr>
<td>Artificial cycle</td>
<td>769 (49.9)</td>
<td>773 (50.1)</td>
<td></td>
</tr>
<tr>
<td>Observed mitosis (cycles, %)</td>
<td></td>
<td></td>
<td>0.0000c</td>
</tr>
<tr>
<td>No</td>
<td>201 (34.1)</td>
<td>388 (65.9)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>932 (54.1)</td>
<td>792 (45.9)</td>
<td></td>
</tr>
<tr>
<td>Damaged blastomere (cycles, %)</td>
<td></td>
<td></td>
<td>0.0000c</td>
</tr>
<tr>
<td>No</td>
<td>932 (53.0)</td>
<td>825 (47.0)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>201 (36.2)</td>
<td>355 (63.9)</td>
<td></td>
</tr>
<tr>
<td>Observed compaction (cycles, %)</td>
<td></td>
<td></td>
<td>0.0000c</td>
</tr>
<tr>
<td>No</td>
<td>819 (45.3)</td>
<td>991 (54.8)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>314 (62.4)</td>
<td>189 (37.6)</td>
<td></td>
</tr>
<tr>
<td>Presence of blood on catheter (cycles, %)</td>
<td></td>
<td></td>
<td>0.000c</td>
</tr>
<tr>
<td>No</td>
<td>678 (55.2)</td>
<td>551 (44.8)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>455 (42.0)</td>
<td>629 (58.0)</td>
<td></td>
</tr>
</tbody>
</table>

\( ^{a}P < 0.05 \) (t-test).
\( ^{b}P < 0.05 \), (Mann–Whitney U-test).
\( ^{c}P < 0.05 \) (x\(^2\) analysis).
Table II  Multivariate logistic regression analysis of factors related to clinical pregnancy of VET.

<table>
<thead>
<tr>
<th>The factors</th>
<th>B</th>
<th>SE(b)</th>
<th>Wald $\chi^2$</th>
<th>P-value</th>
<th>$b'$</th>
<th>ORs (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at COH (years)</td>
<td>$-1.2929$</td>
<td>$0.6374$</td>
<td>$4.1145$</td>
<td>$0.0425$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The reason for freezing (a versus c)</td>
<td>$-0.0553$</td>
<td>$0.0151$</td>
<td>$13.405$</td>
<td>$0.0003$</td>
<td>$-0.0966$</td>
<td>$0.946$</td>
</tr>
<tr>
<td>The reason for freezing (b versus c)</td>
<td>$0.1411$</td>
<td>$0.2116$</td>
<td>$0.445$</td>
<td>$0.5047$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrial thickness</td>
<td>$0.1325$</td>
<td>$0.0309$</td>
<td>$18.41$</td>
<td>$&lt;0.0001$</td>
<td>$0.1143$</td>
<td>$1.142$</td>
</tr>
<tr>
<td>Damaged blastomere</td>
<td>$-0.3927$</td>
<td>$0.1201$</td>
<td>$10.697$</td>
<td>$0.0011$</td>
<td>$-0.0928$</td>
<td>$0.675$</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>$0.2714$</td>
<td>$0.0861$</td>
<td>$9.9432$</td>
<td>$0.0016$</td>
<td>$0.0877$</td>
<td>$1.312$</td>
</tr>
<tr>
<td>No. of good-quality embryos</td>
<td>$0.3844$</td>
<td>$0.0548$</td>
<td>$49.289$</td>
<td>$&lt;0.0001$</td>
<td>$0.1955$</td>
<td>$1.469$</td>
</tr>
<tr>
<td>Presence of blood on catheter</td>
<td>$-0.6005$</td>
<td>$0.0958$</td>
<td>$39.302$</td>
<td>$&lt;0.0001$</td>
<td>$-0.1654$</td>
<td>$0.549$</td>
</tr>
</tbody>
</table>

$B$ regression coefficient; $SE(b)$ standard errors of regression coefficient; $b'$ standardized regression coefficient; a, b, c means the reason of freezing: a, surplus embryos after fresh ET; b, ovarian hyperstimulation syndrome; c, others.

The modified Hosmer–Lemshow goodness-of-fit $\chi^2$ test statistics was 9.8864 ($P = 0.2731$).

Figure 1 ORs and 95% CIs for multivariable analysis of VET.

![Figure 1 ORs and 95% CIs for multivariable analysis of VET.](https://academic.oup.com/humrep/article-abstract/28/7/1768/611035 by guest on 11 April 2019)

Figure 2 Comparisons of ROCs in multivariable analysis.

![Figure 2 Comparisons of ROCs in multivariable analysis.](https://academic.oup.com/humrep/article-abstract/28/7/1768/611035 by guest on 11 April 2019)

(v) damaged blastomere; (vi) number of embryos transferred; (vii) number of good-quality embryos and (viii) presence of blood on catheter.

Table II shows the statistics from the multivariable model. The modified Hosmer–Lemshow goodness-of-fit $\chi^2$ test statistics was 9.8864 ($P = 0.2731$), which suggested that the multivariable model was a good fit ($P > 0.05$). For the reason for freezing, the OHSS group showed a high OR than the other groups (OHSS versus Other, OR: 2.145; CI: 1.4–3.286; Surplus embryos versus Other, OR: 1.152; CI: 0.761–1.743). AH also showed a high OR (OR: 2.105, CI: 1.554–2.85).

Three variables (age at COH, damaged blastomere and presence of blood on catheter) had an adverse effect on the outcome of VET ($b < 0$). Figure 1 shows the ORs and 95% CIs for the multivariable analysis. Figure 2 shows a ROC curves of the multivariable analysis. Table III shows the marginal and partial PEVs of eight variables in the multivariable model. In this analysis $P$-values test the difference from the relative importance of number of good-quality embryos, thus $P$-values of $>0.05$ indicate parameters likely to be useful predictors. The number of good-quality embryos showed the highest marginal PEV and partial PEV (marginal PEV 3.91%, partial PEV 2.28%). The reason for freezing showed the second highest marginal PEV (2.77%). However, number of embryos transferred showed the lowest marginal PEV and partial PEV (marginal PEV 0.21%, partial PEV 0.46%).

Discussion

The successful implantation relies on the hatching of the blastocyst from the ZP. In theory, the freeze–thaw process might further exacerbate hardening of the ZP, which may impair successful embryonic hatching and implantation (Carroll et al., 1990). Although it has not been specifically demonstrated, some randomized studies have investigated that AH performed on vitrified–thawed embryos may assist the natural hatching process to improve the implantation rates (IRs) (Gabrielsen et al., 2004; Balaban et al., 2006; Valojerdi et al., 2008). In contrast, some RCTs were in disagreement with the results on
Table III Variables selected by a bootstrapping stepwise variable selection algorithm associated with successful clinical pregnancy after VET.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PEV marginal (%)</th>
<th>P-value</th>
<th>PEV partial (%)</th>
<th>P-value</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>11.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at COH</td>
<td>0.48</td>
<td>0.0001</td>
<td></td>
<td>0.64</td>
<td>0.0232</td>
</tr>
<tr>
<td>The reason for freezing</td>
<td>2.77</td>
<td>0.2911</td>
<td></td>
<td>1.68</td>
<td>0.5232</td>
</tr>
<tr>
<td>AH</td>
<td>1.97</td>
<td>0.0741</td>
<td></td>
<td>1.02</td>
<td>0.0841</td>
</tr>
<tr>
<td>Endometrial thickness</td>
<td>0.57</td>
<td>0.0001</td>
<td></td>
<td>0.82</td>
<td>0.0588</td>
</tr>
<tr>
<td>Damaged blastomere</td>
<td>2.07</td>
<td>0.0487</td>
<td></td>
<td>0.47</td>
<td>0.0179</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>0.21</td>
<td>&lt;0.0001</td>
<td></td>
<td>0.46</td>
<td>0.0142</td>
</tr>
<tr>
<td>No. of good-quality embryos</td>
<td>3.91</td>
<td>0.0397</td>
<td></td>
<td>2.28</td>
<td>0.6114</td>
</tr>
<tr>
<td>Presence of blood on catheter</td>
<td>1.71</td>
<td></td>
<td></td>
<td>1.81</td>
<td>0.5979</td>
</tr>
</tbody>
</table>

PEV, proportion of explained variation; AUC, area under the receptor operator characteristic curve.

P-values: the difference from the relative importance of number of good-quality embryos.

the positive effect (Primi et al., 2004; Ng et al., 2005; Sifer et al., 2006; Debrock et al., 2011).

It is difficult to assess the value of AH in cryopreserved embryos by one-factor analysis. Many different AH methods were described in the literature, these include laser technology, acid Tyrode solution and mechanically expanding the ZP. The depth and length of zona thinning were diverse in different laboratories. The variation of clinical procedure may influence the outcome, such as endometrial preparation protocol (natural and artificial cycle), the day of freezing/thawing, post-thaw culture duration period and the proportion of VET performed after OHSS. The demographic patient characteristics, sample size and study design can also affect the results. To obviate such potential drawbacks, an independent center with a large sample size of multivariable analysis would provide new insights into the true value of AH. In this study, AH showed a high OR (Table II), demonstrating a significant positive association with clinical pregnancy after VET.

The reason for freezing was ranked as the second most important factor related to clinical pregnancy of VET (Table II). According to the data of this study, the reason for freezing appears to be an important factor related to clinical pregnancy after VET.

Previous studies found that the pregnancy potential of frozen embryos from overstimulated cycles at risk of OHSS is equal to or possibly even superior to that of standard thawed ET (Pattinson et al., 1994; Queenan et al., 1997). At a (2008) reported that the subsequent VET in overstimulated patients with total embryo cryopreservation resulted in an 80% clinical pregnancy rate and 40% live birth rate.

Importantly, before the 'standard thawed ET', most of the patients had a chance to achieve a fresh transfer with their best-quality embryos. Thus, the 'standard thawed ET' was carried out for those patients who failed to conceive in their fresh cycle and a small number who achieved fresh conception but miscarried. Early studies showed that the VET was prone to achieve inferior results in women who failed to conceive in their fresh cycle (Toner et al., 1991; Lin et al., 1995). Subsequent studies with a similar cryo-survival rates and number of embryos showed that VET achieved significantly higher clinical pregnancy and IRs in women who conceived in fresh cycles and in women at risk of OHSS compared with women who failed to conceive in their fresh cycle (El-Toukhy et al., 2003a,b; Urman et al., 2007). While some studies concluded that this was due to better embryo quality (Lin et al., 1995; Urman et al., 2007), the data presented here disagree with that conclusion. Either with the same number of embryos transferred or with the same number of good-quality embryos transferred, the clinical pregnancy rate of the ‘OHSS’ groups was superior to the ‘Surplus’ and ‘Others’ groups (Figs 3 and 4).

Except for OHSS and surplus embryos the reasons for embryo cryopreservation in this study included fluid in the uterine cavity (48 cycles), hydrosalpinx (29 cycles), abnormal endometrium (16) and other marital problems (27 cycles). Recent studies showed that endometrial cavity fluid could have a negative impact on the outcome of IVF-ET, which was associated with hydrosalpinx, polycystic ovarian disease and sub-clinical uterine infections (Griffiths et al., 2002; Akman et al., 2005; He et al., 2010). As for abnormal endometrium, the factors affecting the growth of endometrium are still not well understood. There are many studies that have used exogenous estrogen, low-dose aspirin, etc. to improve endometrial thickness, but there is no agreement in the literature about a consensus treatment (Chen et al., 2006; Khairy et al., 2007; Gleicher et al., 2011).
view of the negative impact on successful outcome in IVF, hydrosalpinx would cause failure of VET without an integrated management (Chanelles et al., 2011; Thalluri and Tremellen, 2012). Even though these adverse factors were detected before the fresh cycle was completed they are difficult to resolve and may still have some impact on the outcome of VET.

Our results are in agreement with those studies that have concluded that the outcome of frozen--thawed ET cycles was dependent on the reason for freezing (OHSS versus Supplement) and the outcome of the fresh cycle from which embryos were derived (El-Toukhy et al., 2003a,b; Urman et al., 2007). Therefore, the reason for freezing seems to be an independent and important factor related to clinical pregnancy outcome of VET.

In conclusion for the Day 3 vitrified--warmed embryos, AH had a positive effect on clinical pregnancy of VET in a multivariable analysis. Other two factors, the number of good-quality embryos and the reason for freezing were the most correlated with clinical pregnancy outcome of VET. As this study was retrospective analysis of data obtained from over 5-year period, a prospective study is needed to further confirm our findings.

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Authors’ roles

W.S. designed the study, drafted and revised the manuscript; X.X. performed data analysis with SAS statistical software; S.Z., W.Z. and M. W. performed all data collections; H.W., H.B. contributed to interpretation of data; J.S. supervised the study and revised the manuscript. All the authors read and approved the final version to be submitted for publication.

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Conflict of interest

None declared.

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