Taiwanese Journal of Obstetrics & Gynecology 54 (2015) 541–545

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Ectopic pregnancy following *in vitro* fertilization with embryo transfer: A single-center experience during 15 years

Ling-Yun Cheng [a], Pin-Yao Lin [b], Fu-Jen Huang [b], Fu-Tsai Kung [b], Hsin-Ju Chiang [b], Yu-Ju Lin [a], Kuo-Chung Lan [b,*]

[a] Department of Obstetrics and Gynecology, Chiayi Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Chiayi, Taiwan
[b] Department of Obstetrics and Gynecology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan

ARTICLE INFO

Article history:
Accepted 18 March 2015

Keywords:
bladder distention
ectopic pregnancy
embryo transfer
in vitro fertilization

ABSTRACT

Objective: Ectopic pregnancy is an obstetrical disease that is potentially associated with maternal death in the first trimester. It is one of the well-known complications following *in vitro* fertilization (IVF) with embryo transfer (ET). The incidence of ectopic pregnancy is estimated to be 2.1–8.6% of clinical pregnancy after IVF-ET, which is higher than natural conceptions (incidence rate 2%). This study aimed to re-evaluate the ectopic pregnancy rate in patients undergoing IVF-ET and to investigate the effects of embryo stage and frozen–thawed blastocyst transfer and ET during full bladder distention on ectopic pregnancy rate.

Materials and methods: This retrospective study reviewed women who achieved a clinical pregnancy after IVF-ET at the Department of Obstetrics and Gynecology, Kaohsiung Chang Gung Memorial Hospital between 1999 and 2013. We compared ectopic pregnancy rate following Day 3 ET with Day 5 ET, and after fresh ET with thawed ET. Besides, multivariate analysis was used to clarify the factors affecting ectopic pregnancy after IVF-ET.

Results: Of the total 1213 clinical pregnancies after fresh ET, 18 (1.5%) were verified as ectopic, which is similar to the rate following natural conception. The ectopic pregnancy rates were similar for Day 3 (1.2%) and Day 5 (1.7%) ETs. The incidence of ectopic pregnancy in thawed ET cycles (0.6%) was not significantly reduced than fresh ET cycles (1.5%). Tubal ET (TET) and ET under full bladder distention had a significant effect on ectopic pregnancy.

Conclusion: Thawed ET was not associated with a lower incidence of ectopic pregnancy than fresh ET, and embryo stage did not affect the rate of ectopic pregnancy. In addition, TET and ET under conditions of full bladder distention may increase the ectopic pregnancy rate.

Introduction

In vitro fertilization (IVF) with embryo transfer (ET) has been reported to result in a higher rate of ectopic pregnancies than spontaneous pregnancies. Approximately 2.1–8.6% of all clinical pregnancies after IVF-ET have been reported to be ectopic [1–3], compared with an ectopic pregnancy rate of 2% after natural conception [4]. Several hypotheses have been advanced to explain this difference, including different hormonal milieu, the reproductive health characteristics of infertile women, technical aspects of IVF procedures, and the estimated embryo implantation potential [2]. Because of the low incidence of ectopic pregnancy, the definite risk factors for ectopic pregnancy after IVF-ET remain inconclusive. Nonetheless, several studies have assessed the risk factors for ectopic pregnancy after IVF-ET with a view to improving IVF-ET outcomes and reducing ectopic pregnancy rates. Although a review of various technical aspects of ET procedures suggested an optimal method of ET [5], no direct relationships between ET techniques and ectopic pregnancy rates have been observed to date.

Over the last few decades, marked improvements in IVF-ET technologies have made treatment courses more similar to

* Corresponding author. Department of Obstetrics and Gynecology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Number 123, Ta-Pei Road, Niao-Sung District, Kaohsiung 833, Taiwan.
E-mail address: lankuochung@gmail.com (K.-C. Lan).

http://dx.doi.org/10.1016/j.tjog.2015.08.004

natural processes. The Society for Assisted Reproductive Technology (SART) reported that the incidence of ectopic pregnancy following IVF-ET declined from 2.1%/clinical pregnancy in 2000 to 1.8%/clinical pregnancy in 2001 [6,7], suggesting that the risk of ectopic pregnancy is no higher after IVF-ET than after natural conception.

Theoretically, blastocyst ET, which is more similar to the natural cycle than cleavage-stage ET, has a higher implantation potential [8]. Previous studies have shown that decreased uterine contractility during the later luteal phase [9] and the larger sizes of blastocysts would prevent the retrograde passage of embryos [10], which imply that blastocyst ET reduces the rate of ectopic pregnancy compared with cleavage-stage ET. In practice, however, the ectopic pregnancy rate was found to be significantly higher after blastocyst ET [11,12]. These results support Chang and Suh's observation that transfer of three or more embryos with higher estimated embryo implantation potential was reported to be associated with an increased risk of ectopic pregnancy [2]. Moreover, two studies suggested that the rate of ectopic pregnancy was not reduced after blastocyst ET compared with cleavage-stage ET [13,14].

This retrospective cohort study was designed to analyze the incidence of ectopic pregnancy after IVF-ET over the past 15 years in our institution. Rates of ectopic pregnancy were compared following fresh versus frozen ET and following cleavage-stage versus blastocyst transfer. Furthermore, the annual incidence of ectopic pregnancy was determined, as well as whether full bladder distention during ET was associated with ectopic pregnancy risk. To address this issue, multivariate analyses were conducted.

Materials and methods

Study population

This retrospective cohort study included all clinical pregnancies conceived after IVF-ET in the Department of Obstetrics and Gynecology, Kaohsiung Chang Gung Memorial Hospital, Taiwan, between January 1999 and December 2013. A clinical pregnancy was defined as the presence of an intrauterine gestational sac on transvaginal ultrasound or the diagnosis of an ectopic pregnancy. Patients' baseline demographic and clinical characteristics were obtained from their medical records. This study excluded oocyte-recipient cycles. The study was approved by the Institutional Review Board of the Ethics Committee of Chang Gung Memorial Hospital, Kaohsiung, Taiwan.

Clinical and laboratory procedures

During the study period, patients underwent ovarian stimulation, which was achieved by gonadotropins, as well as pituitary suppression by either gonadotropin-releasing hormone (GnRH) agonist or GnRH antagonist. After triggering ovulation, oocyte retrieval was followed 34–36 hours later. Oocytes were inseminated conventionally or by intracytoplasmic sperm injection, and embryos were cultured for 3–6 days, depending on their morphological score on Day 2, which was determined by the number of blastomeres and the degree of fragmentation. For frozen–thawed cycles, blastocysts were cryopreserved on Day 5. All women received natural cycle IVF or clomiphene citrate-stimulated cycle ovarian stimulation (Clomid; Sinphar Pharmaceuticals, Yilan, Taiwan) to prepare the endometrium for thawed ET. Our protocols for controlled ovarian hyperstimulation and laboratory procedures have been described elsewhere [15–18]. During ET, the patient was placed in a lithotomy position and the cervix was exposed using a speculum. ET catheter sets (Labotect, GmbH, Germany) were used for all transvaginal ETs, with a standard transfer volume of 20–30 μL. Several patients underwent tubal ET (TET) between 1999 and 2001. Before the first half of 2010, ET was performed without bladder distention; since 2011, all patients underwent ET with full bladder distention without ultrasound guidance. One year later, ET was performed under transabdominal ultrasound guidance, with or without full bladder distention depending on the uterine position. Starting the day after oocyte retrieval and throughout the luteal phase, all patients received either Crinone 8% gel (90 mg daily; Fleet Laboratories Ltd., Watford, UK) or Utrogestan vaginal capsules (200 mg 4 times daily; Piette International Laboratories, Drogenbos, Belgium) [19]. As part of the controlled ovarian hyperstimulation protocol in our center, urinary concentration of beta subunit of human chorionic gonadotropin was measured 2 weeks after ET, and transvaginal ultrasound was performed at 3–5 weeks to confirm the clinical diagnosis of pregnancy.

Ectopic pregnancies

Ectopic pregnancies after IVF-ET were classified as clinical or verified ectopic pregnancies. A verified ectopic pregnancy was defined as the presence of an extrauterine gestational sac on ultrasound or following surgical intervention, whereas a clinical ectopic pregnancy was defined as the absence of an intrauterine gestational sac with abnormally increased serum human chorionic gonadotropin concentrations.

Statistical analysis

All statistical analyses were performed using SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation, median with interquartile range, or *n* (%). Continuous variables were compared using the Mann–Whitney *U* test, whereas categorical variables were compared using the Chi-square test or Fisher exact test depending on sample size. A multiple logistic regression analysis was performed to assess risk factors of ectopic pregnancy following IVF-ET. All tests for significance were two-tailed, with statistical significance defined as a $p < 0.05$. Transfers of fresh and frozen–thawed embryos were analyzed separately. The incidence of ectopic pregnancy was compared after the transfer of fresh Day 3 and Day 5 embryos and after the transfer of fresh and thawed embryos. Annual incidence of ectopic pregnancy in women undergoing IVF-ET in our center and in Taiwan from 1999 to 2013 was analyzed.

Results

A total 3006 IVF cycles following fresh ETs were studied, including 1711 (56.9%) Day 3 and 1295 (43.1%) Day 5 transfers. Of the 1213 clinical pregnancies, 574 (47.3%) resulted from Day 3 and 639 (52.7%) from Day 5 transfers. The characteristics of these clinical pregnancies are presented in Table 1.

During the study period, there were 22 clinical ectopic pregnancies following fresh IVF-ET, among which 18 were verified sonographically or surgically. Thus, the overall rates of clinical and verified ectopic pregnancy for each clinical pregnancy were 1.8% and 1.5%, respectively. Of the 18 verified ectopic pregnancies, 17 were tubal pregnancies (of these, 2 were heterotopic pregnancies, 1 each after Day 3 and Day 5 transfers) and one was a cesarean scar pregnancy. Two of the tubal ETs resulted in verified ectopic pregnancies in 1999. The ectopic pregnancy rates relative to the number of clinical pregnancies and number of transfer cycles are presented in Table 2.

A total of 154 clinical pregnancies resulted from frozen–thawed ET, with three being verified ectopic pregnancies (1.9%), including

Table 1
Characteristics of women with clinical pregnancies after fresh embryo transfer on Day 3 and Day 5.

Characteristic	Full cohort	Day 3 ($n = 574$)	Day 5 ($n = 639$)
Age (y)	32.9 ± 4.3	33.4 ± 4.3	32.3 ± 4.3
No. of oocytes retrieved	7.5 (4.5–10)	5.5 (3–7)	9.4 (7–11)
No. of oocytes transferred	2.8 (2–3)	2.9 (2–3)	2.7 (2–3)
EM thickness (cm)	1.3 ± 0.3	1.3 ± 0.32	1.3 ± 0.32
E2 on hCG day (μg/mL)	1977.2 (978–2644)	1588.2 (756–2084)	2314.8 (1303–3017)
Infertility diagnosis[a]			
Tubal factor	331 (27.3)	165 (28.7)	166 (26.0)
Uterine factor	192 (15.8)	93 (16.2)	99 (15.5)
Male factor	395 (32.6)	185 (32.2)	210 (32.9)
Ovulation factor	169 (13.9)	71 (12.4)	98 (15.3)
Unexplained	188 (15.5)	81 (14.1)	107 (16.7)
Others	42 (3.5)	21 (3.7)	21 (3.3)

Values are mean ± standard deviation, *n* (%), or median (interquartile range).
E2 = estradiol; EM = endometrial; hCG = human chorionic gonadotropin.
[a] Sum may be greater than 100% because some patients had more than one infertility diagnosis.

Table 2
Rates of ectopic pregnancy after fresh and frozen–thawed embryo transfers.

	n (%)
Fresh transfer	
Clinical EP/cycle	22/3006 (0.7)
Clinical EP/clinical pregnancy	22/1213 (1.8)
Verified EP/cycle	18/3006 (0.6)
Verified EP/clinical pregnancy	18/1213 (1.5)
Tubal pregnancy	17
Heterotopic pregnancy	2
Cesarean scar pregnancy	1
Thawed transfer	
Verified EP/cycle	3/498 (0.6)
Verified EP/clinical pregnancy	3/154 (1.9)
Excluding cervical pregnancy/clinical pregnancy	1/154 (0.6)

Values are *n* (rate).
EP = ectopic pregnancy.

two cervical pregnancies. Both cervical pregnancies occurred due to difficulties in performing ET, requiring either Hegar dilators or extensive manipulation with a second catheter. After the exclusion of these confounders, the verified ectopic pregnancy rate after frozen–thawed ET was 0.6%, which was not significantly reduced than the rate following fresh cycle ET ($p = 0.71$).

Table 3 shows details of the rates of ectopic pregnancy following Day 3 and Day 5 ETs. Clinical ectopic pregnancies were observed after 10 of 574 (1.7%) Day 3 and 12 of 639 (1.9%) Day 5 ETs ($p = 0.86$), and verified ectopic pregnancies after seven of 574 (1.2%) Day 3 and 11 of 639 (1.7%) Day 5 transfers ($p = 0.34$). Among the verified ectopic pregnancies, the mean patient age and mean number of embryos transferred were similar after Day 3 and Day 5 ETs.

Because the ET techniques used in our center changed over time, the multivariate analysis was conducted to clarify the factors affecting ectopic pregnancy following fresh IVF-ET. The result showed that TET ($p = 0.005$) and full bladder distention during ET ($p = 0.010$) had a significant effect on ectopic pregnancy after IVF-ET (Table 4). The annual incidence of verified ectopic pregnancy from 1999 to 2013 is shown in Figure 1. The incidence was significantly higher in 2011 than in the previous years, with four verified ectopic pregnancies occurring during that year. This increase may be due to changes in ET techniques, specifically from ET without bladder distention to ET with full bladder distention.

Table 3
Comparison of ectopic pregnancy rates after Day 3 and Day 5 fresh embryo transfers.

	Day 3	Day 5	*p*
Clinical EP (%)	10 (1.7)	12 (1.9)	0.86
Verified EP (%)	7 (1.2)	11 (1.7)	0.34
Age (y)	29.1 ± 4.5	31.8 ± 6.2	0.47
No. of oocytes transferred	3.0 (2–4)	2.5 (2–3)	0.24

Values are presented as *n* (incidence), mean ± standard deviation, or median (interquartile range).
EP = ectopic pregnancy.

Discussion

Since Steptoe and Edwards [20] first reported ectopic pregnancy following IVF, the epidemiology of and risk factors for ectopic pregnancy after IVF have been widely investigated. In the past, ectopic pregnancy was thought to be a well-known risk following IVF. Differences between natural conception and IVF-ET may affect the incidence of ectopic pregnancy. Despite the health characteristics of infertile women, improvements in IVF-ET technology have made this process more similar to natural conception in the recent decades. Rates of ectopic pregnancy following IVF-ET have been reduced by restricting the number of embryos transferred [1,2], avoiding deep fundal transfer [21], and TET [2], injecting a smaller volume of fluid during ET, and transferring frozen–thawed embryos [22,23]. Over the past 15 years, the verified ectopic pregnancy rate following fresh cycle ET in our center was 1.5% of clinical pregnancies, which is in good agreement with the rate of 1.8% reported by SART in 2001 (1.8%) [6] and similar to the 2% rate observed following natural conception [4]. Thus, our findings confirm that ectopic pregnancy is no longer a complication specifically associated with IVF-ET.

In general, there has been a belief that higher progesterone concentrations in the luteal phase may reduce uterine contractility during fresh ET, which may prevent the embryo from migrating into the fallopian tubes [24]. However, a growing number of studies are now available to shed some light on lower ectopic pregnancy rate following frozen–thawed ET [22,23]. The clinical pregnancy rate was found to be higher following frozen–thawed than fresh ET [25], suggesting impairment of endometrial receptivity after ovarian stimulation in fresh ET. The lower ectopic pregnancy rate after frozen–thawed ET than fresh ET may be due to the negative effect of ovarian stimulation on endometrial receptivity in the latter. However, some studies reported no reduced ectopic pregnancy rates with thawed ET [26,27]. After excluding the two cervical pregnancies, which occurred due to difficulties in performing ET, we found no significant difference in ectopic pregnancy rate between frozen–thawed and fresh ET. Although low incidence of ectopic pregnancy and relative small sample size of frozen–thawed ET may affect the result in our study, our finding was not able to

Table 4
Multivariate analysis of variables in relation to ectopic pregnancy.

Variable	B	SEM	Wald test	*p*
Physician difference	—	—	—	0.114
TET	−3.200	1.128	8.045	0.005
No. of embryo transfer	—	—	—	0.313
Embryo stage on transfer day (Day 3/Day 5)	—	—	—	0.111
Age of female partners (y)	—	—	—	0.052
EM thickness (cm)	—	—	—	0.058
E2 on hCG day (μg/mL)	—	—	—	0.147
Tubal factor infertility	—	—	—	0.138
Ultrasound guidance	—	—	—	0.460
Full bladder distention during embryo transfer	−3.295	1.273	6.697	0.010

B = coefficient; EM = endometrial; E2 = estradiol; hCG = human chorionic gonadotropin; SEM = structural equation modeling; TET = tubal embryo transfer.

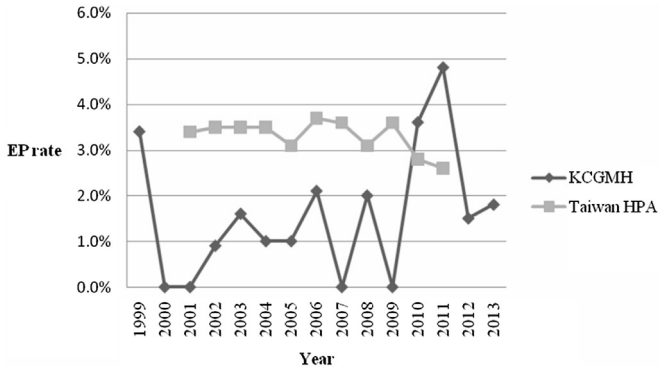

Figure 1. Annual incidence of ectopic pregnancy in women undergoing *in vitro* fertilization with embryo transfer in our center and in Taiwan from 1999 to 2013. EP = ectopic pregnancy; HPA = Health Promotion Administration; KCGMH = Kaohsiung Chang Gung Memorial Hospital.

indicate no difference in the risk of ectopic pregnancy among fresh ET compared with frozen–thawed ET.

There may be a concern for a lower ectopic pregnancy rate following blastocyst transfer as speculated by decreased uterine contractility in the later luteal phase and larger embryo diameter. When not restricted by patient history or number of embryos transferred, however, we found that ectopic pregnancy rates were similar following Day 3 and Day 5 ETs. Another large study, which adjusted for the number of fertilized embryos, found no statistically significant difference in ectopic pregnancy rates between Day 3 and Day 5 ETs [14]. These results support previous research, which suggests that the higher implantation potential at the blastocyst stage may increase the risk of ectopic pregnancies except when only one or two embryos are transferred [2].

Although embryo quality and endometrial receptivity are factors associated with pregnancy rate, ET is regarded as the critical element in the final step of IVF cycles. Since uterine straightening by bladder distention was first shown to be beneficial [28], several studies have investigated the correlations between pregnancy outcomes and full bladder distention during ET. A full bladder helps to straighten the uterocervical angle and facilitates entry of the catheter, especially for the anteverted uterus. A systematic review summarized that passive full bladder distention during ET catheter placement may optimize the outcomes of ET without having direct adverse effects, including multiple pregnancies and miscarriages [29]. To date, however, no study has analyzed the relationship between full bladder distention and ectopic pregnancy rates. In the first half of 2010, the ET techniques in our center were modified, from ET without bladder distention to ET with full bladder distention. This was accompanied by a significant increase in the ectopic pregnancy rate in 2010 and 2011. These findings suggested that a full bladder may straighten not only the uterocervical angle but also the utero-fallopian angle. The utero-fallopian angle in the patient with anteverted uterus had larger angles under no bladder distention compared with full bladder distention. This indicated that full bladder distension makes the uterus and the fallopian tubes lie nearly on the same plane, allowing embryos to more easily migrate into the fallopian tubes. After the introduction of ultrasound-guided ET in 2012, ectopic pregnancy rates have decreased. ET under ultrasound guidance helps physicians evaluate the uterocervical angle and decide whether their patients have full bladder distention or not. Furthermore, it avoids deep fundal transfer. Despite the impact of new strategy with ultrasound-guided ET, the result of our study suggests that changing from no to full bladder extension may have increased the ectopic pregnancy rate. However, this proposal is an intuitive hypothesis, requiring more empirical investigation.

This study is unique in competitive case numbers of Day 3 and Day 5 ETs in our center, thus reducing possible statistical bias. However, this study had limitations, including its retrospective design and the relatively low rate of ectopic pregnancy. There were also several potential confounders, including tubal disease; numbers of oocytes retrieved, embryos fertilized, and embryos transferred/cycle; and ET strategies. Moreover, this retrospective study covers a long period, and may thus pose the risk of bias if the IVF protocol changes over this interval.

Conclusions

Three of our findings are worth summarizing. First, with the significant improvements in IVF technology, ectopic pregnancy is no longer a complication specifically associated with IVF-ET. Second, the embryo stage on the day of transfer did not affect the ectopic pregnancy rate. Finally, the strategy used for ET may affect the incidence of ectopic pregnancy. Specifically, in addition to TET, we found that the ectopic pregnancy rate was associated with the extent of bladder distention. The relationship between ectopic pregnancy and bladder distention requires further investigation.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

The authors would like to thank Dr Scott Butler, PhD, for English editing and Yun-Fang Chiang, RN, of the Department of Obstetrics and Gynecology at Chang Gung Memorial Hospital for assistance in patient registration and data collection.

References

[1] Clayton HB, Schieve LA, Peterson HB, Jamieson DJ, Reynolds MA, Wright VC. Ectopic pregnancy risk with assisted reproductive technology procedures. Obstet Gynecol 2006;107:595–604.

[2] Chang HJ, Suh CS. Ectopic pregnancy after assisted reproductive technology: what are the risk factors? Curr Opin Obstet Gynecol 2010;22:202–7.

[3] Nazari A, Askari HA, Check JH, O'Shaughnessy A. Embryo transfer technique as a cause of ectopic pregnancy in *in vitro* fertilization. Fertil Steril 1993;60:919–21.

[4] Centers for Disease C, Prevention. Ectopic pregnancy—United States, 1990–1992. MMWR Morb Mortal Wkly Rep 1995;44:46–8.

[5] Mains L, Van Voorhis BJ. Optimizing the technique of embryo transfer. Fertil Steril 2010;94:785–90.

[6] Society for Assisted Reproductive Technology, American Society for Reproductive Medicine. Assisted reproductive technology in the United States: 2001 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology registry. Fertil Steril 2007;87:1253–66.

[7] Society for Assisted Reproductive Technology, American Society for Reproductive Medicine. Assisted reproductive technology in the United States: 2000 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. Fertil Steril 2004;81:1207–20.

[8] Glujovsky D, Blake D, Farquhar C, Bardach A. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev 2012;7:CD002118.

[9] Fanchin R, Ayoubi JM, Righini C, Olivennes F, Schonauer LM, Frydman R. Uterine contractility decreases at the time of blastocyst transfers. Hum Reprod 2001;16:1115–9.

[10] Schoolcraft WB, Surrey ES, Gardner DK. Embryo transfer: techniques and variables affecting success. Fertil Steril 2001;76:863–70.

[11] Keegan DA, Morelli SS, Noyes N, Flisser ED, Berkeley AS, Grifo JA. Low ectopic pregnancy rates after *in vitro* fertilization: do practice habits matter? Fertil Steril 2007;88:734–6.

[12] Rosman ER, Keegan DA, Krey L, Liu M, Licciardi F, Grifo JA. Ectopic pregnancy rates after *in vitro* fertilization: a look at the donor egg population. Fertil Steril 2009;92:1791–3.

[13] Milki AA, Jun SH. Ectopic pregnancy rates with day 3 versus day 5 embryo transfer: a retrospective analysis. BMC Pregnancy Childbirth 2003;3:7.

[14] Smith LP, Oskowitz SP, Dodge LE, Hacker MR. Risk of ectopic pregnancy following day-5 embryo transfer compared with day-3 transfer. Reprod Biomed Online 2013;27:407–13.

[15] Ou YC, Lan KC, Huang FJ, Kung FT, Lan TH, Chang SY. Comparison of *in vitro* fertilization versus intracytoplasmic sperm injection in extremely low oocyte retrieval cycles. Fertil Steril 2010;93:96–100.

[16] Lan KC, Hseh CY, Lu SY, Chang SY, Shyr CR, Chen YT, et al. Expression of androgen receptor co-regulators in the testes of men with azoospermia. Fertil Steril 2008;89:1397–405.

[17] Lan KC, Huang FJ, Lin YC, Kung FT, Hsieh CH, Huang HW, et al. The predictive value of using a combined Z-score and day 3 embryo morphology score in the assessment of embryo survival on day 5. Hum Reprod 2003;18:1299–306.

[18] Lin P-Y, Huang F-J, Kung F-T, Wang L-J, Chang SY, Lan K-C. Comparison of the offspring sex ratio between fresh and vitrification-thawed blastocyst transfer. Fertil Steril 2009;92:1764–6.

[19] Wang LJ, Huang FJ, Kung FT, Lin PY, Chang SY, Lan KC. Comparison of the efficacy of two vaginal progesterone formulations, Crinone 8% gel and Utrogestan capsules, used for luteal support in blastocyst stage embryo transfers. Taiwan J Obstet Gynecol 2009;48:375–9.

[20] Steptoe PC, Edwards RG. Reimplantation of a human embryo with subsequent tubal pregnancy. Lancet 1976;1:880–2.

[21] Pope CS, Cook EK, Arny M, Novak A, Grow DR. Influence of embryo transfer depth on *in vitro* fertilization and embryo transfer outcomes. Fertil Steril 2004;81:51–8.

[22] Ishihara O, Kuwahara A, Saitoh H. Frozen-thawed blastocyst transfer reduces ectopic pregnancy risk: an analysis of single embryo transfer cycles in Japan. Fertil Steril 2011;95:1966–9.

[23] Shapiro BS, Daneshmand ST, De Leon L, Garner FC, Aguirre M, Hudson C. Frozen-thawed embryo transfer is associated with a significantly reduced incidence of ectopic pregnancy. Fertil Steril 2012;98:1490–4.

[24] Pyrgiotis E, Sultan KM, Neal GS, Liu HC, Grifo JA, Rosenwaks Z. Ectopic pregnancies after *in vitro* fertilization and embryo transfer. J Assist Reprod Genet 1994;11:79–84.

[25] Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for *in vitro* fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. Fertil Steril 2011;96:344–8.

[26] Check JH, Choe JK, Katsoff B, Krotec JW, Nazari A. Ectopic pregnancy is not more likely following fresh vs frozen embryo transfer. Clin Exp Obstet Gynecol 2005;32:95–6.

[27] Jun SH, Milki AA. Ectopic pregnancy rates with frozen compared with fresh blastocyst transfer. Fertil Steril 2007;88:629–31.

[28] Sundstrom P, Wramsby H, Persson PH, Liedholm P. Filled bladder simplifies human embryo transfer. Br J Obstet Gynaecol 1984;91:506–7.

[29] Abou-Setta AM. Effect of passive uterine straightening during embryo transfer: a systematic review and meta-analysis. Acta Obstet Gynecol Scand 2007;86:516–22.