RESEARCH ARTICLE

Frozen embryo transfers can reduce the risk of ectopic pregnancy in patients with non-tubal factor infertility undergoing elective freeze-all procedures

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OBJECTIVES: The aim of this study was to compare the risk of ectopic pregnancy (EP) after Fresh and frozen embryo transfer in non-tubal infertility cases.

METHODS: The patients were grouped into three according to the nature of the embryo transfer as Fresh and frozen ET with surplus embryos from a previous fresh (sFET) and frozen ET with embryos from a previous freeze-all cycles (eFET). Cases included in these groups were further categorized according to the day of uterine replacement. Differences between the intrauterine and EP groups were compared with the use of generalized estimation equations (GEEs) with a link function.

RESULTS: A total of 13.261 cycles were analyzed and EP occurred in 1.6%, 1%, and 0.9% of clinical pregnancies after fresh ET, sFET, and eFET respectively ($p<0.05$). EP was found to be 1.4% among day 3 ETs and was 1.3 % among day 5 ETs, which was not statistically significant in fresh and frozen ET cycles ($p<0.05$). Moreover, transferring embryos either on day 3 or day 5 was found to result in similar EP rates in fresh ET and sFET groups. In the eFET group, day 3 transfer strategy was found to has significantly decreased EP rates ($p<0.05$). Among day 5 embryo transfers, EP rates displays similar values in fresh and FET groups that have been transferred on day 5, whereas for day 3 transfers, the risk of EP has been shown to be significantly decreased in eFET group ($p<0.01$)

CONCLUSION: Results of this study indicate that, frozen embryo transfers can increase overall cycle outcome by increasing the clinical pregnancy rates and reducing the EP rates. Although both day 3 and day 5 embryo transfers can confer a similar EP risk, embryos in day 3 eFET cycles displayed the lowest risk for EP.

Key Words: *Ectopic pregnancy; Frozenembryo; eFET; Elective freeze all*

INTRODUCTION

Ectopic pregnancy (EP), defined by the heterotopic implantation of an embryo other than the intrauterine cavity, is a serious cause of first trimester maternal mortality [1]. Recent studies imply that there is an increased risk of EP following assisted reproduction technologies (ART) [2]. However, it is still controversial that increased risk of EP following ART can be due to the effects of the treatment or the resulting effects of the underlying diagnosis.

The incidence of EP is estimated to be 1-2% of all natural conceptions and the primary ethology includes all types of tubal pathology like tubal obstruction, inflammation and functional abnormalities. Although the embryo is directly transferred into the uterine cavity during IVF/ICSI treatment, the incidence of EP still varies between 2.1 and 8.6% after ART [3,4].

Among all patient-related risk factors, tubal factor infertility (TFI) is currently known to be the leading factor for EP, whose occurrence can be as high as 11% among TFI patients [4]. Recent studies have considered three main issues, namely the nature of ET strategy, the day of uterine ET and the quality of embryos transferred as the major risk factors for EP during ART [5-8], Extending the day of ET through blastocyst-stage theoretically seems to decrease the incidence of EP, however, a number of recent studies indicated that, there is no significant difference in terms of EP risk between day 3 and day 5 ET [9-11]. Possible embryonic factors such as chromosomal abnormalities and abnormal expression of adhesion factors during implantation have been questioned as a potential cause of EP during ART [12,13].

This study aimed at comparing the risk of EP after Fresh ET and FET in non-tubal infertility cases. Theoretically, fresh ET includes the 'best' embryo and FET after fresh ET refers to the 'second best qualified embryo transfers; however e-FET cycles refer to the 'best embryo transfers in a more natural hormonal mileniu. As the quality of the transferred embryo is one of the suggested risk factor for EP, clinical outcome in FET cases was also sub-grouped according to the treatment strategy that has been sought for frozen embryo transfers, namely, FET with surplus embryos after Fresh ET (sFET), or elective FET after a freeze-all cycle (eFET).

MATERIALS AND METHODS

The present research is a retrospective cohort study, which was conducted at Bahceci Health Group between March 2010 and November 2014. The study was approved by Bilim University (4414529/2015-61) Ethical Committee. Cycles with tubal factor aetiology and cases in which tubal patency were not confirmed and excluded.

For analysis, patients were first grouped into three according to the nature of the embryo transfer as i) Fresh ET, ii) frozen ET with surplus embryos from a previous fresh ET cycle (sFET) and iii) frozen ET with embryos from a previous freeze-all cycle (eFET) respectively. Cases included in these groups were further categorized according to the day of transfer.

In all cycles, controlled ovarian stimulation, Oocyte retrieval, denudation, ICSI and vitrification, and warming procedures were performed as described in detail previously by Serdarogullari and colleagues [14]. Endometrial preparation for eFET and sFET involved hormone replacement therapy and luteal phase support was continued until the 10th week of pregnancy.

Serum HCG was initially measured to determine pregnancy after 12 days of embryo transfer. A second hCG test was taken 48 hours after the initial measurement in order to monitor he expected doubling on the hormone level as an indicator of intrauterine and EP. Intrauterine pregnancy was

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defined as the presence of a gestational sac with fetal heart rate. An EP was defined when TV-USG revealed an extrauterine gestational sac.

Patient characteristics are presented as mean, standard deviation (SD), and the causes of infertility in the intrauterine and EP groups by transplantation group are presented as n (%). Differences between the intrauterine and EP groups were compared with the use of generalized estimation equations (GEEs) with a link function, a linear regression model or a binary logistic regression model for continuous variables and categorical variables, respectively. All statistical analyses were performed with the use of SPSS 18.0 statistics software, and values of P<0.05 were considered to indicate statistical significance.

RESULTS

From March 2010 to November 2014, 16,423 fresh and frozen ET cycles were performed in Bahceci Umut and Fulya IVF Centers. A total of 3162 cycles were excluded from the analysis group due to the presence of tubal factor ethology or ambiguous tubal patency status.

In total, 13,261 ET cycles were included in the study. Of these, 7156 (54%) ET were performed as fresh ET, 2450 (18.5%) of these cycles were sFET and 3655 (27.5%) of them were performed as eFET cycles respectively.

The aetiology of infertility for these cycles were male factor (n=2652; 20%), ovulatory dysfunction (n=1458; 11%), endometriosis (n=604; 4.6%) unexplained (n=2254; 17%), male and female factor combined (n=3.780; 28.5%), diminished ovarian reserve (n=2513; 18.9%). Demographical characteristics and clinical pregnancy outcome is shown in Table 1.

TABLE 1
Study groups and their cycle outcomes with respect to the type of embryo transfer performed

Study groups	Fresh ET	sFET	eFET	p value
Female age (mean ± SD)	34.1 ± 7.3	32.8±5.0	34.1 ± 5.6	p<0.01
ET cycles (n)	7156	2450	3655	-
BMI(kg/m2) (mean ± SD)	24,5 (± 5.2)	24,3 (± 5.1)	24 (± 5.9)	0.41
Reason for infertility				0.12
Endometriosis	707 (9.9%)	229 (9.3%)	352 (9.6%)	-
DOR	937 (13.1%)	320 (13.1%)	477 (13%)	-
PCOS	717 (10%)	242 (9.9%)	367 (10%)	-
Male factor	1649 (23%)	549 (22.4%)	851 (23.3%)	-
Combined male/ female factors	1314 (18.4%)	505 (20.6%)	677 (18.5%)	-
Unexplained	1832 (25.6%)	605 (24.7%)	931 (25.5%)	-
CPR (%)	38.4	48.0	47.0	p<0.01
Total clinical pregnancies (n)	2750	1177	1717	-
Intrauterine pregnancies (n)	2705	1165	1701	-
Ectopic pregnancies n, (%)	45 (1.6)	12 (1.0)	16 (0.9)	p<0.05

During the study period, a total of 73 (1.3%) EPs were diagnosed. The mean age among women with EP was 34.3 ± 5.3 years. Of these 73 patients, 17 (23.3%) had male factor, 12 (16.4%) had ovulatory dysfunction, 14 (19.2%) had unexplained infertility (tubal factor was excluded by hysterosalphingography), 12(16.5%) had diminished ovarian reserve, 17 (23.2%) had male and female factor, 1 (1.4%) had endometriosis. In cases of male and female factor; female factor was composed of 12 (16,5%) ovulatory dysfunction, 4 (5,6%) diminished ovarian reserve and 1(1,4%) endometriosis.

Overall, EP rate was found to be 1.4% (18/1315) among day 3 ETs and was 1.3 % (55/4329) among day 5 ETs, which was not statistically significant in fresh and frozen ET cycles (p>0.05). When study groups have been sub grouped and analysed with the day of embryo transfer, transferring embryos either on day 3 or day 5 was found to result in similar EP rates in fresh ET and sFET groups. In the eFET group, day 3 transfer strategy was found to has significantly decreased EP rates (p<0.05) (Table 2).

Table 2
Subgroup analysis according to the day of embryo transfer

	ET cycles (n)	CPR (%)	Total clinical pregnancies (n)	Intrauterine pregnancies (n)	Ectopic pregnancies n, (%)	p value
Fresh ET						
Day 3	3257	26.4	860	844(98.1)	16(1.9)	-
Day 5	3899	48.5	1890	1861(98.5)	29(1.5)	NS
sFET						
Day 3	340	27.9	95	94(98.9)	1(1.1)	-
Day 5	2110	51.3	1082	1071(99)	11(1)	NS
eFET						
Day 3	1473	24.4	360	359(99.7)	1(0.03)	-
Day 5	2182	62.2	1357	1342(98.9)	15(1.1)	p<0.01

Note: Difference of ectopic pregnancy rate between groups was compared with the use of generalized equation estimating equations with a binary logistic regression model link function.

Among day 5 embryo transfers, EP rates displays similar values in fresh and FET groups that have been transferred on day 5, whereas for day 3 transfers, the risk of EP has been shown to be significantly decreased in eFET group (p<0.01) (Table 3). Patient characteristics, day of embryo transfer and transfer group's variables were used to identify which of them could affect the EP. Day 3 ET(OR: 1.11; 95% CI 1.08-1.17) and fresh (OR:1.07; 95% CI 1.05-1.1) and FET (OR:1.08; 95% CI 1.06-1.11) were found to effect EP.

Table 3
Analysis of study groups according to the day of embryo transfer

	ET cycles (n)	CPR (%)	Total clinical pregnancies (n)	Intrauterine pregnancies (n)	Ectopic Pregnancies n, (%)	P value
Day 3 uterine replacements						
Fresh ET	3257	26.4	860	844	16(1.9)	NS
sFET	340	27.9	95	94	1(1.1)	NS
eFET	1473	24.4	360	359	1(0.3)	p<0.01
Total	5070	25.9	1315	1297	18 (1.4)	-
Day 5 uterine replacements						
Fresh ET	3899	48.5	1890	1861	29(1.5)	NS
sFET	2110	51.3	1082	1071	11(1)	NS
eFET	2182	62.2	1357	1342	15(1.1)	NS
Total	8191	52.8	4329	4274	55 (1.3)	-

Note: Difference of ectopic pregnancy rate between groups was compared with the use of generalized equation estimating equations with a binary logistic regression model link function.

DISCUSSION

Tubal factor infertility, cigarette smoking and endometriosis have been found to be highly associated with the risk of EP in the general population. Although it is expected to be lower than the ratio in spontaneous conceptions, the risk of EP has also been found to be more common in IVF/ICSI cycles, albeit the underlying causes are still unknown [15].

Changes in the endocrine millieu during COH have been proposed to be a major risk factor for the development of EP in ART. There may also be significant contributions on EP with the embryo development and embryo transfer technique-related issues [6-11,16-18]. In this study, by excluding tubal factor infertility which had already been found to be associated with elevated EP rates, we have tried to focus on the type and the day of ET.

Our results have confirmed the findings of the previous reports, compared to fresh ETs, FET cycles generates decreased risk of EP (Table 1). These results also support the hypothesis that COH can create a uterine environment that can lead to an abnormal implantation. The possible explanation for the higher EP rates is supraphysiologic hormonal levels and oocyte retrieval procedure caused disorganized uterine contractility may lead to the intrauterine placed embryos to enter and implant on the tubes [19,20]. The other explanation is secondary tubal dysfunction due to a supraphsiologic hormonal effect [21]. Female age in the sFET group was found to be slightly younger than the others; however, such a finding can be attributed to the fact that young patients generate more embryos that can be frozen as surplus. This slight but significant difference is also not expected to create a bias in the statistics as indicated in a previous study [22].

We have investigated if there is a significant difference on the risk of EP with respect to the day of embryo replacement. A cumulative analysis and comparison in the study population did not find a significant difference in EP rates between day 3 and day 5 embryo transfers respectively. This result was found to be contradictory with Fang et al. [5]; however it could be due to the fact that TFI cases were excluded in this analysis.

The same data set have been further compared the cycle outcome according to the day of embryo transfer within each group. Comparison of relative EP rates on day 3 have resulted in relatively reduced EP rates in sFET and eFET groups as compared to Fresh ET group and the difference was statistically significant for eFET group ($p<0.05$). For day 5 ETs, although EP rate was found to be higher in the Fresh ET group, no statistically significant difference was found among the Fresh and FET groups respectively.

Reduced EP rates in sFET and eFET groups undergoing day 3 ET corroborate with the results of Shapiro and colleagues [8] in such a way that, transferring embryos with high implantation potential can create a similar selection effect as day 5 ET after day 3 warming and extended culture to blastocyst stage. In the eFET group, both the benefit of extended culture and improved receptivity in terms of frozen embryo replacement could create an environment that reduces the risk of EP. Besides eFET cycles in which morphologically best-graded embryos were transferred after warming, it is generally expected that morphologically inferior embryos were usually utilized during sFET procedure. Since it is known that the quality of the embryo is a suggested risk factor for EP, a poor quality embryo may have a low eutopic implantation potential and may increase the risk of EP [23]. Such a hypothesis could in fact create another potential difference between sFET and eFET results in terms of EP rates.

It is reported that the direction of the uterine contractility are from cervix to fundus and frequency and amplitude decreases following hCG trigger 6 or 7 days later [19]. Hence, in theory, fresh Day 5 ETs could lower the EP rates in fresh cycles. However, in our study no significant difference was observed in Fresh Day 3 ETs and Fresh Day 5 ETs. This results support previous researches that also reported similar EP rates after Fresh day 3 and day 5 transfers [9-11].

CONCLUSION

In conclusion, to our knowledge, this is the first study to analyse the EP rates in Fresh ET, sFET and eFET cycles involving non-TFI cases. EP is a serious early pregnancy complication, confers emotional and physical risk and increases treatment costs. In our study, frozen embryo transfers can reduce EP rates. Although both day 3 and day 5 embryo transfers can confer a similar EP risk, embryos in day 3 eFET cycles have been observed to have the lowest risk for EP in our study population. Additional, prospective, cohort studies are needed to better evaluate eutopic, ectopic and heterotopic implantation rates.

ETHICAL CONSIDERATIONS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study

CONFLICT OF INTEREST

All the authors declare that there is no conflict of interest.

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