Risk factors for Ectopic Pregnancy Following In-Vitro Fertilization in a Tertiary Hospital in the Philippines

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Objectives: To determine the incidence of ectopic pregnancy after IVF at the Center for Advanced Reproductive Medicine and Infertility (CARMI) in St. Luke's Medical Center-Global City (SLMC-GC) and to describe the clinical characteristics of these patients and identify possible risk factors for ectopic pregnancy following IVF.

Study Design. This is a descriptive cross-sectional study, with the digital database of CARMI as the source of patient information. Demographic and clinical data were obtained and tabulated. Statistical analyses were performed to identify possible risk factors for the development of ectopic pregnancy in IVF cases.

Results. There were a total of 243 clinical pregnancies among the 929 IVF-ET cases from September 2011 to April 2015. There were five cases of ectopic pregnancy. Frozen embryo transfer was the only variable found to be significantly associated with ectopic pregnancy (p=0.003).

Conclusion. The ectopic pregnancy rate among IVF cycles in CARMI is 2.1%. Only frozen embryo cycle was found to be significantly associated with the development of EP. The other clinical variables that were studied showed no statistical significance.

Key words: ectopic pregnancy, frozen embryo transfer, IVF

Introduction

Ectopic pregnancy (EP) is an abnormal pregnancy wherein the fertilized ovum implants outside the intrauterine cavity, with the ampulla of the fallopian tube being the most common site.[1] It occurs in 1-2% of all natural pregnancies and the incidence increases after assisted reproductive techniques (ART).[2] In vitro fertilization (IVF) is considered a major predisposing factor for the development of EP, with two to three folds increased risk compared to the general population.[3] The prevalence of EP following ART ranges between 2.1 to 8.6% of all pregnancies and it can reach up to 11% in women with a history of tubal factor infertility.[4]

The increased risk of EP following fertility treatment may be due to the effects of the treatment or the pre-existing cause of infertility.[2,5] Factors related to the cause of female infertility and IVF procedure have been identified as risk factors for EP. With the increasing trend of IVF practice in the Philippines, it would be beneficial to assess the local incidence and compare the risk factors identified in foreign studies with local data. And knowing that hemorrhage due to tubal rupture is

the most common cause of maternal mortality in the first trimester of pregnancy,[6] early prognostication is vital.

Objectives

1. To determine the incidence of ectopic pregnancy after IVF at the Center for Advanced Reproductive Medicine and Infertility (CARMI) in St. Luke's Medical Center – Global City (SLMC-GC).

2. To describe the clinical characteristics of patients and identify possible risk factors who had ectopic pregnancy following IVF at CARMI in SLMC-GC, in terms of the following clinical variables:
   - o Maternal age
   - o Weight
   - o Race or ethnicity
   - o History of smoking
   - o Infertility diagnosis
   - o Value of AFC, AMH, FSH, LH and E_2
   - o Year of ART procedure
   - o Previous ectopic pregnancy
   - o Previous surgery
   - o Presence of endometriosis
   - o Presence or history of pelvic inflammatory disease (PID)
   - o Presence of uterine abnormalities
   - o FSH dosage
   - o Type of trigger
   - o Type of luteal phase support
   - o Use of assisted hatching
   - o Use of ICSI
   - o Fresh or frozen ET
   - o Day of ET
   - o Number of embryos transferred
   - o Number of embryos cryopreserved
   - o ET Technique (catheter, depth of ET, use of tocolytic)

Materials and Methods

Study Design and Population

This is a descriptive cross-sectional study approved by the Institutional Scientific Review Committee and the Institutional Ethics Review Committee. We reviewed the ART charts of all women with clinical pregnancies after IVF at the Center for Reproductive Medicine and Infertility (CARMI) in St. Luke's Global City from September 2011 to April 2015.

The digital database of CARMI was the source of information for this study. The investigators evaluated the completeness of the said database. The following clinical data were extracted: maternal age, body mass index (BMI), infertility diagnosis, year of ART procedure, history of pelvic inflammatory disease (PID), type of trigger, use of laser assisted hatching, use of intracytoplasmic injection, fresh or frozen ET, day of ET, number of embryos transferred, number of embryos cryopreserved, type of catheter used in ET, use of atosiban, uterine depth of ET and pregnancy status.

Clinical intrauterine pregnancy was confirmed by ultrasound showing a gestational sac within the uterus.[7] Ectopic pregnancy was diagnosed as having a gestational sac is implanted outside of the uterus (as seen on ultrasound) or rising beta hCG in the absence of an intrauterine gestational sac, or presence of a complex adnexal mass.[7,8]

Data analysis

Data were processed and analyzed using SPSS 16, and were interpreted using independent t-test for comparison of continuous variables and chi-square test for comparison of categorical variables. Multiple logistic regression using backward stepwise method was used for identification of risk factors at 5% level of significance. Odds ratios and corresponding 95% confidence intervals will be presented.

Results

There were a total of 929 cases of IVF with subsequent ET in CARMI, from September 2011 to April 2015. Of which, 246 resulted to a clinical pregnancy. Three of these clinical pregnancies have incomplete data. Table 1 shows the distribution of cases, according to the type of pregnancy. The ectopic pregnancy rate is 2.1%

(n= 5). Of the 238 (97.9%) cases of intrauterine pregnancies, 40 cases (16.5%) resulted in miscarriages. There were 149 (61.3%) singleton pregnancies and 49 (20.2%) multiple gestation cases.

Table 1. Type of pregnancy distribution.

Type of Pregnancy	Frequency	Percent
Pregnancy		
Ectopic	5	2.1
Miscarried	40	16.5
Singleton	149	61.3
Multiple	49	20.2

Table 2 shows the mean age, BMI, number of prior ART cycles, day of transfer, number of embryos transferred, number of embryos cryopreserved, and depth of transfer both in the ectopic and intrauterine group. The mean values for maternal age, day of transfer, number of embryos transferred, number of embryos cryopreserved and the depth of transfer are similar for both groups. There was no statistically significant difference between the two groups in terms of the abovementioned variables.

Table 3 shows the infertility diagnoses in the two groups. There was no significant difference noted between the ectopic and the intrauterine group when it comes to the cause of infertility.

Table 2. Possible risk factors between ectopic and intrauterine group.

Factors	Ectopic		Normal		t-test
	Mean	SD	Mean	SD	p value
Maternal age	34.6	5.5	34.3	4.4	0.871
BMI	27.1	10.7	23.9	3.4	0.654
Number of prior ART cycles	0.5	1.0	0.2	0.5	0.640
Day of transfer	3.3	1.5	3.2	1.0	0.774
Number of embryos transferred	3.6	0.5	2.9	0.8	0.063
Number of embryos cryopreserved	2.0	1.9	2.0	2.6	0.986
Depth of transfer	9.2	2.8	10.6	3.4	0.383

Table 3. Infertility diagnosis between ectopic and intrauterine group.

Infertility Diagnosis	Ectopic		Normal		P value
	Frequency	Row %	Frequency	Row %	
Sex factor					0.626
Male	0	0.0	43	100.0	
Female	2	1.9	102	98.1	
Mixed	0	0.0	41	100.0	
Unexplained fertility	0	0.0	6	100.0	
Advanced age					0.292
Yes	1	2.6	38	97.4	
No	1	0.6	153	99.4	
Ovulatory dysfunction					0.351
Yes	0	0.0	58	100.0	
No	2	1.5	133	98.5	
Endometriosis					0.279
Yes	1	2.6	37	97.4	
No	1	0.6	154	99.4	
Tubal					0.511
Yes	0	0.0	34	100.0	
No	2	1.3	157	98.7	

Infertility Diagnosis	Ectopic		Normal		P value
	Frequency	Row %	Frequency	Row %	
Adenomyosis					0.884
Yes	0	0.0	2	100.0	
No	2	1.0	189	99.0	
Immunologic					0.858
Yes	0	0.0	3	100.0	
No	2	1.1	188	98.9	
Endometrial polyp					0.884
Yes	0	0.0	2	100.0	
No	2	1.0	189	99.0	
Myoma					0.918
Yes	0	0.0	1	100.0	
No	2	1.0	190	99.0	
Poor or decreased ovarian reserve					0.817
Yes	0	0.0	5	100.0	
No	2	1.1	186	98.9	
Uterine					0.884
Yes	0	0.0	2	100.0	
No	2	1.0	189	99.0	
Vaginismus					0.918
Yes	0	0.0	1	100.0	
No	2	1.0	190	99.0	
Azoospermia					0.61
Yes	0	0.0	22	100.0	
No	2	1.2	169	98.8	
Asthenozoospermia					0.836
Yes	0	0.0	4	100.0	
No	2	1.1	187	98.9	
Idiopathic oat					0.407
Yes	0	0.0	49	100.0	
No	2	1.4	142	98.6	
Oligospermia					0.918
Yes	0	0.0	1	100.0	
No	2	1.0	190	99.0	
Prostate cancer					0.918
Yes	0	0.0	1	100.0	
No	2	1.0	190	99.0	
Tetratospermia					0.836
Yes	0	0.0	4	100.0	
No	2	1.1	187	98.9	
Varicocoele					0.918
Yes	0	0.0	1	100.0	
No	2	1.0	190	99.0	
Sexual dysfunction					0.918
Yes	0	0.0	1	100.0	
No	2	1.0	190	99.0	
Reversal					0.918
Yes	0	0.0	1	100.0	
No	2	1.0	190	99.0	
Unexplained infertility					0.799
Yes	0	0.0	6	100.0	
No	2	1.1	185	98.9	

Table 4 shows the other variables that may cause ectopic pregnancies such as: history of pelvic inflammatory disease (PID), type of ovulation trigger, use of assisted hatching, use of ICSI, type of embryo transferred, type of catheter used for ET, use of stylet during ET, and use of atosiban. Only the type of embryo transfer was found to be clinically significant (p=0.003). Those who had frozen IVF had increased risk of ectopic pregnancy (11.8%) compared to those who had fresh IVF (1.3%).

Discussion

Ectopic pregnancy is a known risk of IVF. The rate of ectopic pregnancy is higher in pregnancies resulting from ART, with incidence ranging from 2% to as high as 11% compared to only 1-2% in spontaneous pregnancies. In this study, the computed EP among IVF cycles in CARMI is 2.1%, which is the same with the EP rate in the general population. This is low compared to the higher EP rates presented by other studies and this can be explained by the relatively low number of IVF cases in this study compared to larger international studies as the center has only been operating for 5 years.

Tubal damage was also found to be the primary cause of EP in IVF, which is also the most common cause of EP in spontaneous pregnancies.[3] Tubal abnormalities, most likely secondary to pelvic inflammatory disease, results from alterations in

Table 4. Other possible risk factors for ectopic pregnancy between ectopic and intrauterine group.

Infertility Diagnosis	Ectopic		Normal		P value
	Frequency	Row %	Frequency	Row %	
PID					0.576
Yes	0	0.0	26	100.0	
No	2	1.2	166	98.8	
Laser Hatching					0.236
Yes	3	3.5	82	96.5	
No	2	1.3	156	98.7	
ICSI					
Yes	5	2.1	238	97.9	
No	0	0.0	0	0.0	
Trigger					0.259
Buserelin	1	3.0	32	97.0	
Recombinant HCG	1	6.7	14	93.3	
Urinary HCG	1	0.9	111	99.1	
Embryo transferred					0.003
Fresh	3	1.3	223	98.7	
Frozen	2	11.8	15	88.2	
Use of Atosiban					0.476
Yes	5	2.3	216	97.7	
No	0	0.0	22	100.0	
Type of catheter					0.632
Kita-zato	0	0.0	2	100.0	
Labotect	0	0.0	35	100.0	
Wallace	5	2.4	201	97.6	
Stylet					0.316
Yes	5	2.5	198	97.5	
No	0	0.0	40	100.0	

tubal transport mechanisms and expression of molecules that normally inhibit blastocyst implantation in the fallopian tube.[9, 10,11] However, tubal damage is not the only pathology that explains EP in ART. There can also be abnormalities in endometrial receptivity with ectopic implantation occurring after failure of the normal biological interactions between endometrium, fallopian tube and embryo due to the controlled ovarian stimulation that is utilized in ART and the subsequent alteration in hormonal milieu that is intended during an IVF cycle.[3] In this present study, both tubal factor as cause of infertility and history of PID were not found to be associated with increased risk for EP.

Of all the possible risk factors assessed in this study, only frozen embryo transfer was found to be significantly associated with ectopic pregnancy ($p = 0.0003$). This was the same finding of the groups of Pyrgiotis, Kashyap and Silva, et al.[12,13,14] They explained the higher risk of EP among those who had frozen ET through different mechanisms. First would be the lower progesterone level compared with the supraphysiologic level of progesterone in a fresh cycle, which renders the uterus to be more relaxed, preventing migration of the embryo to the fallopian tube. Second would be the developmental delay of thawed embryos, which may lead to a longer lag time before implantation in the uterus, increasing the opportunity for the movement of the embryo towards the extrauterine space. Lastly uterine dimensions could be different depending on the ovarian stimulation. Ovarian hyperstimulation leading to elevated estrogen levels may cause increase in the uterine dimension, larger than in frozen cycle. There could be a tendency to transfer embryos to the same depth during fresh and frozen embryo transfers, resulting in the injection of embryos closer to the fallopian tubes.

The significant association between frozen ET and EP is in contrast with the result of the systematic review of Perkins et al on the risk of EP associated with ART in the US. They found the highest rate of EP among fresh cycles. This can be explained by elevated hormone levels seen during ovarian stimulation used in fresh cycles that can alter the uterine environment during embryo transfer, causing increased uterine contractility, which may result in retrograde movement of the embryo into the fallopian tube.[8] This was also the same finding by the group of Shapiro et al that performed a retrospective cohort study on 2,150 cases of embryo transfers. They noted a significantly decreased risk of EP among those who underwent frozen ET compared to fresh transfers (0.3% versus 2.5%). On the other hand, the result of a systematic review by Acharya, et al on EP rates in frozen versus fresh ET showed no statistical significance in the rates of EP between frozen and fresh ET. The result of this present study on the significant association between frozen embryo transfer and ectopic pregnancy could be explained by the small number of ectopic pregnancy (N=5), two of which were frozen embryo transfers thus the noteworthy association.

Other known risk factors for EP such as maternal age, non-modifiable risk factor for EP with the highest incidence seen in the 35- to 44-year age group, was not associated with EP in this present study. Advanced maternal age increases the risk for EP by the accumulation of risk factors over time as a woman ages and changes in the anatomy and function of the fallopian tube that may predispose the embryo to implant at extrauterine site.

Embryos that have undergone assisted hatching have been reported to implant earlier compared with unhatched embryo, therefore has a higher risk for EP. In the present study, assisted hatching was not found to increase the risk for EP. This was the same finding of Hagemann, et al on their prospective randomized study that evaluated the effect of assisted hatching on pregnancy outcomes of IVF cycles. They found no association between assisted hatching and EP.[15]

Transfer of a blastocyst stage embryo has been implicated with a higher risk for EP compared to a cleavage stage embryo because of the general higher implantation rate of blastocyst stage embryo, but this was not observed in this study.[3]

The risk of EP in cases wherein three or more embryos had been transferred was 2.4 – 2.5% compared to only 1.4% when less than 2 embryos are transferred. This is according to the study of Chang and Suh.[16] This correlation was not seen in

our study, even if the mean number of embryo transferred in the ectopic group is 3.6, and only 2.9 in the intrauterine group.

The distance from the fundal endometrium to the air bubble after ET has also been investigated as a potential risk factor for EP. Coroleu, et al. reported that the placement of the transfer catheter close to the fundus (within 10 mm) resulted in EP while a distance of 15–20 mm from the fundus achieved higher implantation and pregnancy rates. [17] In this study, both the mean of depth transfer for both groups was less than 10 mm, thus there was no significant association noted.

This study did not find any significant association with EP and the following variables: maternal BMI, number of prior ART cycles, number of embryos cryopreserved, use of ICSI, type of ovulation trigger, use of atosiban, type of catheter and use of stylet in ET. A limitation of this study is the relatively small number of women with ectopic pregnancy, which is mainly due to a small number of IVF cycles.

Conclusions and Recommendations

The ectopic pregnancy rate among IVF cycles in CARMI, St. Luke's Global City is 2.1%. This is same with the worldwide incidence of EP not only in ART cycle, but also in the general population.

Only frozen embryo cycle was found to be significantly associated with the development of EP. The other clinical variables that were studied showed no statistical significance.

Future studies having a more robust study population and carried for a longer period of time would give more objective results with regards to factors causing EP after IVF procedures in our local setting.

References

1. Shaw JL, Diamandis EP, Horne AW, Barnhart K, Bourne T, Messinis IE. Ectopic pregnancy. Clin Chem 2012; 58: 1278-85.
2. Jurkovic D, Wilkinson H. Diagnosis and management of ectopic pregnancy. BMJ 2011; 342: d3397.
3. Refaat B, Dalton E, Ledger W. Ectopic pregnancy secondary to in vitro fertilisation-embryo transfer: pathogenic mechanisms and management strategies. Reproductive Biology and Endocrinology 2015; 13: 30.
4. 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.
5. Sivalingam VN, Duncan WC, Kirk E, Shephard LA, Horne AW. Diagnosis and management of ectopic pregnancy. J Fam Plann Reprod Health Care 2011; 37: 231-40.
6. Khan KS, Wojdyla D, Say L, Gulmezoglu AM, Van Look PF. WHO analysis of causes of maternal death: a systematic review. Lancet 2006; 367: 1066-74.
7. Perkins KM, Boulet SL, Kissin DM, Jamieson DJ. Risk of Ectopic Pregnancy Associated with Assisted Reproductive Technology in the United States, 2001-2011. Am Coll Obstet Gynecol 2015; 125(1).
8. Perkins KM, Boulet SL, Kissin DM, Jamieson DJ. Risk of Ectopic Pregnancy Associated with Assisted Reproductive Technology in the United States, 2001-2011. Am Coll Obstet Gynecol 2015; 125(1).
9. Shaw JL, Dey SK, Critchley HO, Horne AW. Current knowledge of the aetiology of human tubal ectopic pregnancy. Hum Reprod Update 2010; 16: 432-44.
10. Refaat B. Role of activins in embryo implantation and diagnosis of ectopic pregnancy: a review. Reprod Biol Endocrinol 2014; 12: 116.
11. Refaat B, Simpson H, Britton E, Biswas J, Wells M, Aplin JD, et al.Why does the fallopian
12. Pyrgiotis E, Sultan KM, Neal GS, et al. Ectopic pregnancies after in vitro fertilization and embryo transfer. J Assist Reprod Genet 1994; 11: 79-84.
13. Kashyap S, Chung P, Kligman I, et al. 7 year descriptive summary of ectopic pregnancies occurring after fresh and frozen IVF cycles [abstract]. Fertil Steril 2002; 78: S137.
14. Silva C, Trimachi J, Keefe D, Frankfurter D. High incidence of ectopic pregnancy following frozen embryo transfer [abstract]. Fertil Steril 2003; 80 (Suppl 3):S178.
15. Hagemann AR, Lanzendorf SE, Jungheim ES, et al. A prospective, randomized, double-blinded study of assisted hatching in women younger than 38 years undergoing in vitro fertilization. Fertil Steril 2010; 93: 586-91.
16. Chang HJ, Suh CS. Ectopic pregnancy after assisted reproductive technology: what are the risk factors. Curr Opin Obstet Gynecol 2010; 22: 202-7.
17. Coroleu B, Barri PN, Carreras O, Martinez F, Parriego M, Hereter L, et al. The influence of the depth of embryo replacement into the uterine cavity on implantation rates after IVF: a controlled, ultrasound-guided study. Hum Reprod 2002; 17(2): 341-6.