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Research Article

Analytical Study with Fresh and Frozen Embryos and the Incidence of Ectopic Pregnancy in *In vitro* Fertilization

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Abstract

Objective: To analyze the rates of ectopic pregnancies (EP) in *in vitro* fertilization using fresh and frozen embryos.

Design: Retrospective study of all transfers of fresh and frozen embryos from January 2007 to December 2014.

Setting: Institutional IVF center.

Patients: A total of 933 patients undergoing *in vitro* fertilization.

Intervention: Fresh and frozen embryos obtained through *in vitro* fertilization via intracytoplasmic sperm injection.

Main outcome measures: The rates of Ectopic pregnancy in both groups.

Results: Of the 933 embryos obtained through *in vitro* fertilization that resulted in clinical pregnancies, 19 cases of ectopic pregnancies were observed, a prevalence of 2.02%. Fresh embryos led to 772 fertilizations, and 161 were the product of frozen embryos. Using the fresh embryos, 16 ectopic pregnancies occurred, 2.1% of fertilizations; with frozen embryos, the rate was 1.9%. There was a lower rate of ectopic pregnancy with frozen embryos in comparison to fresh embryos, although this did not demonstrate statistical significance (p=0.86) (OR=0.89, CI=0.258-3.11).

Conclusion: The profile of patients with ectopic pregnancy was young patient with young partner that showed good ovarian response, male factor as major indication for the procedure followed by tubal factor, and had more than 3 embryos transferred into the middle of the uterus. The prevalence of EP in this study was 2.02. No significant difference was observed in EP when the transfer of fresh and frozen embryos was compared.

Keywords: Ectopic pregnancy; Fresh embryos; ICSI; IVF

Introduction

In recent years there has been a significant increase in the number of frozen embryo transfers in comparison with fresh embryos. From 2006 to 2012 there was an 82.5% increase in transfers of frozen embryos and only 3.1% for fresh embryos, as reported by the Society for Assisted Reproductive Technology (SART). From 2011 to 2012 there was a 17.3% increase in frozen embryos and a 3.2% increase in fresh embryos, showing the growth in use of frozen embryos [1].

Initially, cryopreservation was used to complement *in vitro* fertilization (IVF) techniques. In 1954 the first published cryopreservation of sperm occurred [2]. The first birth resulting from frozen embryos took place in 1984, and the first birth from frozen oocytes was in 1996 [3,4].

Currently, clinical indications for cryopreservation include: cancer patients undergoing treatment, patients at risk for premature ovarian failure, and freezing to delay fertility, a result of the current tendency for women to have children at more advanced ages [4].

The rates of live birth increased significantly with the use of frozen embryos compared with fresh embryos. It is known that implantation depends on three parameters: the quality of the embryo, the receptivity of the endometrium, and the interaction between them [5,6]. In fertilization with frozen embryos, endometrial preparation is improved, and there is a consequent improvement in responsiveness when compared with cycles done with fresh embryos [5,7,8]. Rates of ectopic pregnancy in IVF vary from 2.0% to 8.6% of all clinical pregnancies; while the rates range from 0.3% to 1.97% in spontaneous gestations [9,10]. However, in groups with tubal commitment, these rates can be higher, up to 11% [11].

There is still controversy regarding the rates of ectopic pregnancies for fresh and frozen embryos. Some studies suggest that the rates of ectopic pregnancies are higher for frozen embryos than fresh embryos; these studies suggest that the supra-physiological concentration of progesterone produced by multiple corpora lutea combined with exogenous supplementation produces greater concentrations than in frozen embryos; keeping the uterus more relaxed [12-14].

With the increase in transfers of frozen embryos and studies in the literature showing divergent rates of ectopic pregnancies, the objectives of this study were to define the profile of patients with ectopic pregnancy, establish the prevalence of ectopic pregnancy in high complexity ICSI *in vitro* procedures, and to compare the rates of EP in IVF for fresh and frozen embryos [15,16].

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Materials and Methods

This study was approved by The Institutional Review Board of Plataforma Brazil, and owing to the retrospective nature the requirement of informed consent was waived. This was a retrospective study of all transfers of fresh and frozen embryos conducted from January 2007 to December 2014 were done to compare the rates of ectopic pregnancy. We reviewed all transfers of fresh and frozen embryos obtained through *in vitro* fertilization via intra-cytoplasmic sperm injection conducted between January 2007 and December 2014. Clinical pregnancies included pregnancies where the embryonic sac could be seen intrauterally as well as ectopic pregnancies. Ectopic pregnancies were diagnosed by ultrasound or laparoscopy.

The patients underwent ovulation induction with pituitary and ovarian stimulation using recombinant gonadotropins, the dose of which was calculated according to the patient's profile. The patients were followed to monitor ovulation in the third and sixth day, and used the GnRH antagonist (Orgalutran/Organon) when follicles 14 mm in size were present. The dosage of FSHR (Gonal 450/Merck) was adjusted according to ovarian response. The patients were monitored using ultrasound until two follicles were present with a mean diameter of 18 mm. On this day, appropriate dating was conducted, and subcutaneous HCG-r (Ovidrel 250/Merck) was initiated for follicular maturation and puncture. After collection, micronized vaginal progesterone 200 µcg (Utrogestan/Besins) three times a day was initiated.

After ICSI was performed on the selected embryos, they were transferred on the third day using a Frydman Soft transfer catheter with mandrel (Laboratoire CCD, Paris, France) guided by abdominal ultrasound. After the transfer was completed, the patients were kept in a resting position for 30 min and instructed to maintain bed rest for 24 h. After this period, the patients were permitted to return to their usual activities.

The freezing technique used was vitrification (vitrification kit Ingámed - cryopreservation of oocytes in metaphase II and embryos in the zygote stage to blastocysts). For the frozen embryo transfer, the patients were started on estradiol valerate 2 mg (Primogyna/Bayer) once daily for 4 days, then 2 mg twice daily for 4 days, and then 2 mg three times a day. On the thirteenth day, micronized progesterone 200 mcg was begun every 8 h. Subsequently, ultrasound was performed to assess the endometrium, considering a thickness of more than 7 mm as appropriate. The frozen embryo transfer took place on the third day of progesterone.

The records reviewed were stored as electronic health records in the Ultrasystem program, version 3.8.1.The profile of the patients with ectopic gestations was evaluated with respect to age of patient, age of partner, number of follicles collected, number of M2 follicles, transfer location, indication for the procedure, and number of embryos transferred. The prevalence of ectopic pregnancy and the comparison of rates between the groups of fresh and frozen embryos were conducted via logistic regression analysis using SPSS version 20.0 software.

Results

Among the 933 embryos obtained through vitro fertilization that resulted in clinical pregnancies, 19 cases of ectopic pregnancies were observed.

The mean age of the 19 patients presenting ectopic pregnancy was 33.4 years, and the mean partner age was 36.07 years. As for the number of follicles collected, 52.63% of the patients had more than 11 follicles and 63.15% of patients had more than 7 M2 follicles, showing good ovarian response (Tables 1 and 2). With respect to the number of embryos transferred, 31.57% of patients had more than 4 embryos transferred and 52.63% had 3 embryos transferred.

Among the clinical indications, male factor infertility was present in 6 patients, tubal issues isolated were present in 4 patients, infertility without apparent cause was present in only 1 patient, the other indications were: endometriosis in 1 patient, persistent thin endometrium in 1 patient, and ovarian factors in 3 patients (Table 3).

More than one cause of infertility was present in 3 patients. In these patients with more than one cause of infertility, the first one showed tubal factor associated with male factor, the second one presented with male factor, tubal factor and endometriosis, and the third one showed tubal factor and endometriosis (Table 3).

With regard to the location of intra-uterine transfer in the 19 patients, in 12 cases the embryos were transferred into the middle of the uterus, and 3 were transferred into lower segment of the uterus. No complications occurred during these transfers. A total of 772 fertilizations occurred using fresh embryos (group I) and 161 with frozen embryos (group 2). Of the 933 *in vitro* fertilizations, 19 ectopic pregnancies occurred, a prevalence of 2.02%. In group I, 16 ectopic pregnancies occurred, a rate of 2.1%; in group 2, the rate was 1.9%. There was a lower rate of ectopic pregnancy with frozen embryos in comparison to fresh embryos, although this did not demonstrate statistical significance (p=0.86) (OR=0.89, CI 0.258-3.11) (Table 4).

Number of follicles	Absolute Number	Relative Number
≤ 5	2	10.52%
6-10	7	35.84%
≥ 11	10	52.63%
Total	19	100.00%

Table 1: Distribution of ectopic pregnancy cases following IVF (Fértile, Goiânia 2007-2014) according to the number of follicles collected.

Number of M2 Follicles	Absolute Number	Relative Number
≤ 3	3	15.78%
4-6	2	10.52%
7-9	8	42.10%
≥ 10	4	21.05%
Not reported	2	10.50%
Total	19	100.00%

Table 2: Distribution of ectopic pregnancy cases following IVF (Fértile, Goiânia 2007-2014) according to the number of M2 follicles.

Indication	Absolute Number	Relative Number
Male factor	6	31.57%
Tubal Factor	4	21.05%
Ovarian Factor	3	15.78%
More than 1 factor	3	15.78%
No apparent cause	1	5.26%
Endometriosis	1	5.26%
Persistently thin endometrium	1	5.26%
Total	19	100%

Table 3: Distribution of ectopic pregnancy cases following IVF (Fértile, Goiânia 2007-2014) according to clinical indication.

	Fresh embryos		Frozen embryos		b	P	OR	IC	
	N	%	N	%				Inferior	Superior
Normal	756	97.9	158	98.1	-0.109	0.864	0.897	0.258	3.116
Ectopic	16	2.1	3	1.9					
Total	772	100	161	100					

Table 4: Comparing ectopic pregnancies among groups of fresh and frozen embryos (Fértile, Goiânia 2007-2014).

Discussion

Infertility is a universal phenomenon that affects approximately 8% to 15% of couples, and is associated with a higher risk of ectopic pregnancy [17]. Even after *in vitro* fertilization, when the embryos are transferred directly into the uterine cavity, there is an increase in the prevalence of ectopic pregnancy [18]. This rate ranges from 2.0-8.6% of all clinical pregnancies, and is 0.3-1.97% in spontaneous pregnancies [9,10]. This study found an overall prevalence of 2.02%, similar to the rates found in the literature.

In recent years, lifestyle changes have led to a significant increase in demand for IVF techniques, and more recently this increase has been more significant in fertilization using frozen embryos.

There is no agreement in the literature about whether the use of frozen or fresh embryos presents higher risk for ectopic pregnancies. Recent studies show a protective factor against ectopic pregnancy when frozen embryos are transferred [19].

Decleer et al. showed a 1.92% rate of EP when fresh embryos were used and 1.28% when frozen embryos were used, with no statistical significance [17]. In 2011, a Japanese study found that IVF using frozen embryos could result in lower risk of EP in patients, and deserves further study [16]. Shapiro et al. presented statistically significant rates showing lower prevalence of ectopic pregnancy in frozen embryos (0% or 0/433), while for fresh embryos this number was 1.9% (14/725) (p=0.0016) [15].

Huang et al. conducted a study of 30,000 assisted reproductive cycles resulting in 6,431 clinical pregnancies using fresh embryos and 5,564 clinical pregnancies using frozen embryos. The ectopic rate in pregnancies from fresh embryos was 4.62% and was 2.22% for frozen embryos (P<0.001) [20]. Fang et al. conducted a study of 3,183 women who received 3,340 transfers. The rate of ectopic pregnancy in the fresh embryos transferred on day 3 was 2.4%, while for fresh embryos transferred on day 5 the rate was 1.7%. The frozen embryos transferred on day 3 presented a rate of 1.9%, while the frozen embryos transferred on day 5 had a rate of 0.3%, indicating statistical significance [21].

It is believed that this protection derives from improvements in embryo quality as well as endometrial receptivity. The most widely accepted hypothesis is the negative effect of ovarian stimulation on endometrial receptivity. This hypothesis seems to be valid, because a detrimental effect of ovarian stimulation in the endometrium can affect implantation rates and lead to an increase in ectopic pregnancy and a decrease in the rate of intra-uterine pregnancies [15]. This study agrees with the findings of another randomized trial conducted by the same group, which strongly suggests that the rates of ectopic pregnancies declined in stimulated fresh cycles compared with cycles using frozen embryos in normo-responsive patients [19].

In contrast with these authors, a meta-analysis of seven studies including 13,059 pregnancies showed a 2.31% rate in frozen embryos and 1.48% using fresh embryos. These rates did not show statistical differences (OR=1.66, CI 95%, 0.62-4.41) [14]. A study by Jun et al. presented rates of 2.8 and 1.8 for fresh and frozen embryos, respectively [13]. A recent study using the SART database showed that among 103.070 cycles, the clinical pregnancy that resulted in EP were 65% lower in women who had a frozen embryo transfer compared with a fresh embryo transfer in autologous cycles [22].

Limitations of this study include the small sample size and its retrospective nature. The data obtained in this study showed a lower prevalence of ectopic gestation in frozen embryos, without statistical significance. With the improvement in the rates of pregnancies with frozen embryos, recent studies show a tendency towards lower rates of ectopic pregnancy in frozen embryos, but further studies should be conducted since different rates for the two groups are found in the literature.

Conclusion

The profile of patients with ectopic pregnancy was young patient with young partner that showed good ovarian response, male factor as major indication for the procedure followed by tubal factor, and had more than 3 embryos transferred into the middle of the uterus. The prevalence of ectopic pregnancy in this study was 2.02. It was observed that there was no significant difference in ectopic pregnancy compared the transfer of fresh and frozen embryos.

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