Taiwanese Journal of Obstetrics & Gynecology 54 (2015) 567–571

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

The influence of female age on the cumulative live-birth rate of fresh cycles and subsequent frozen cycles using vitrified blastocysts in hyper-responders

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ARTICLE INFO

Article history:
Accepted 16 June 2015

Keywords:
cumulative live-birth rate
female age
vitrified blastocyst

ABSTRACT

Objective: The aim of this research was to study the influence of female age on the cumulative live-birth rate of fresh and subsequent frozen cycles using vitrified blastocysts of the same cohort in hyper-responders.

Materials and methods: This was a retrospective study of 1137 infertile women undergoing their first *in vitro* fertilization treatment between 2006 and 2013. The main outcome measure was cumulative live births among the fresh and all vitrified blastocyst transfers combined after the same stimulation cycle. The results were also analyzed according to age (i.e., <35 years, 35–39 years, and ≥40 years).

Results: The mean number of retrieved oocytes was 19.9 ± 8.5 oocytes. The cumulative pregnancy rate was 89.2% and the cumulative live-birth rate was 73.3%. The cumulative live-birth rate declined from 73.9% for women younger than 35 years old to 67.3% for women 35–39 years old to 57.9% for women 40 years or older.

Conclusion: Combined fresh and vitrified blastocyst transfer cycles can result in a high cumulative live-birth rate. The cumulative live-birth rates among older women are lower than the rates among younger women when autologous oocytes are used.

Introduction

In the beginning of *in vitro* fertilization (IVF) treatment, all available embryos were transferred because of the low success rate; however, improvements in the clinical and laboratory aspects of IVF have increased the pregnancy rate and the risk of multiple pregnancies. To prevent multiple pregnancies, fewer embryos are transferred and the supernumerary embryos are cryopreserved for potential future use [1,2].

After IVF, the cryopreservation of human embryos is more important than ever for the cumulative pregnancy rate. In the past few years, the general success rates for frozen–thawed embryo transfers (FETs) have increased, and a recent systematic review shows that FET results in significantly higher ongoing pregnancy rates, compared to fresh embryo transfer (ET) [3]. According to the United States (U.S.) Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) [4], which collects data on the success rates of assisted reproductive technology (ART) among all American fertility clinics, the live-birth rate in 2012 was 46.9% for fresh cycles and 42.0% for FET cycles [4]. A recent review supports that FET reduces the risk of ovarian hyperstimulation syndrome and improves the outcomes for the mother and the baby [5].

Consistent clinical outcomes similar to those from fresh transfers have been reported after vitrification. A systematic review and meta-analysis showed a significantly higher ongoing pregnancy rate with vitrification, compared to slow freezing [6]. The literature

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http://dx.doi.org/10.1016/j.tjog.2015.08.009

on vitrification has primarily focused on cryopreservation of blastocyst-stage embryos. A Cochrane review demonstrated that live-birth rates can be optimized by performing fresh blastocyst transfers, compared to cleavage stage ETs [7].

The success rate and live-birth rate of IVF demonstrate a similar age-related decline in the chance of a natural pregnancy [8]. Patient-specific parameters such as maternal age can also impact embryo development and competency [9]. Luke et al [10] report that the delivery rates are lower among older women than among younger women when autologous oocytes are used.

Recent publications have reported IVF success rates in cumulative delivery rate per woman, and thus provide a more realistic estimate that is applicable to individual couples [11,12]. This study was designed to assess the influence of female age on the cumulative live-birth rates of fresh cycles and subsequent frozen cycles using vitrified blastocysts of the same cohort.

Materials and methods

Patient selection

We performed a retrospective study of 1137 couples undergoing their first controlled ovarian stimulation for IVF at the Lee Women's Hospital in Taichung, Taiwan between September 2006 and August 2013. Eligibility inclusion criteria were: (1) a woman younger than 42 years old; (2) a woman undergoing her first IVF or intracytoplasmic sperm injection (ICSI) cycle; (3) a long ovulation induction protocol; (4) ejaculated sperm origin; (5) blastocyst transfer in the fresh cycle; and (6) vitrified blastocyst transfer in the frozen cycle. The exclusion criteria were (1) oocyte donation cycles; (2) vitrified oocytes cycles; (3) nonejaculated sperm; (4) two pronucleate or cleavage ET; and (5) lack of a vitrified blastocyst. Ethics approval was obtained from the Institutional Review Board of Chung Shan Medical University Hospital (Taichung, Taiwan). The intention was to study the cumulative live-birth rates that utilized vitrified blastocysts and their fresh siblings from the same stimulation cycle.

Stimulation cycle

Details of the stimulation cycle have been previously reported [13]. In brief, the protocol began with daily subcutaneous injections of leuprolide acetate (Lupron; Takeda Pharmaceutics, Konstantz, Germany) 0.5 mg on Day 21 of the prestimulation cycle. On cycle Days 3–7, gonadotropin (Gonal-F, 225 IU/day; Serono, Bari, Italy) was administered subcutaneously. To stimulate follicular development, the dose was then adjusted according to the ovarian response. When two or more follicles reached a maximum diameter of 18 mm, 10,000 IU human chorionic gonadotropin (hCG; Profasi; Serono) was administered. Transvaginal oocyte retrieval was performed 32–34 hours after the hCG injection. Fertilization was performed by conventional insemination or by ICSI, depending on the semen parameters. The standard of care throughout the study period was blastocyst transfer. Fresh ET was performed with the replacement of at most two blastocysts with the best quality.

Embryo cryopreservation and vitrified ETs

Supernumerary blastocysts were cryopreserved with vitrification. The details of the vitrification and thawing protocols have been previously reported [14]. The vitrified blastocysts were thawed on the morning of the FET, and were discarded if more than 50% of the original blastomeres had degenerated on thawing. Frozen–thawed blastocysts were transferred in natural cycles in ovulatory women or in hormone replacement cycles for anovulatory women. A maximum of two frozen blastocysts were transferred in any one FET cycle. Patients failing to achieve a live-birth after fresh ET went through cryopreserved cycles until all vitrified embryos were transferred or a live-birth was achieved.

Pregnancy outcome

A pregnancy test was performed 14 days after the transfer. Pregnancy was defined by a serum hCG concentration above 10 IU/L. One week later, ultrasound examination was offered to pregnant women to confirm the intrauterine pregnancy. Live births were defined by the birth of at least one live infant. The cumulative live-birth rate was the proportion of transfers that resulted in at least one live birth, whether from the first transfer attempt or subsequent transfers of the frozen–thawed supernumerary blastocysts.

Statistical analysis

The primary outcome measure was the cumulative live-birth rate among the fresh cycles and all FET cycles combined, after the same stimulation cycle. Differences in the pregnancy rate and live-birth rate between the age groups were analyzed using the Chi-square test. Statistical analysis was performed using the Statistical Program for Social Sciences, version 15.0 (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant.

Results

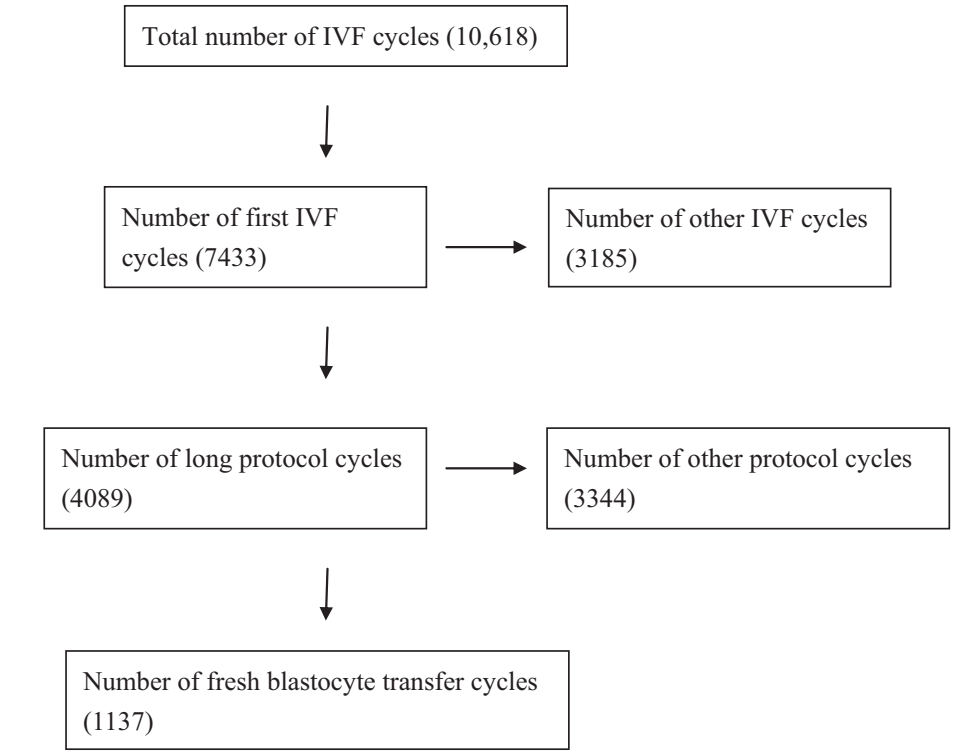
Figure 1 shows the flow chart of the enrollment process for the patients. The characteristics of the study population are presented in Table 1. The final data set for analysis included 1137 women with an initial fresh cycle (i.e., cycle 1). Among these women, 390 women did not become pregnant and underwent a first frozen cycle (i.e., cycle 2). Twenty-nine women did not become pregnant in cycle 2 and underwent a second frozen cycle (i.e., cycle 3). The clinical pregnancy rate per transfer was 65.7% in cycle 1, 64.1% in cycle 2, and 56.8% in cycle 3. The cumulative pregnancy rate was 87.7% after two cycles (i.e., the fresh cycle and the first frozen cycle) and 89.2% after three cycles (i.e., the fresh cycle and two subsequent frozen cycles; Table 2). The live-birth rate was 53.8% in cycle 1, 53.1% in cycle 2, and 48.3% in cycle 3. The cumulative live-birth rate was 72.0% after two cycles and 73.3% after three cycles (Table 3).

The cumulative pregnancy rates among women after two cycles decreased with age (<35 years, 88.5%; 35–39 years, 85.6%; and ≥40 years, 84.2%; Table 4). The cumulative live-birth rates among the women after two cycles significantly decreased with age (<35 years, 73.9%; 35–39 years, 67.3%; and ≥40 years, 57.9%; $p = 0.038$; Table 5). The IVF/ET-related complications were multiple pregnancy and ovarian hyperstimulation syndrome. The estimated rates of these complications were 36.7% and 2.8%, respectively.

Discussion

Our retrospective study shows that IVF-ICSI and vitrified blastocysts offer high cumulative pregnancy rates (89.2%) and cumulative live-birth rates (72.3%) with the same cohort oocytes. Optimization of stimulation protocols and laboratory techniques has contributed to overall greater success rates and a greater number of high-quality embryos to cryopreserve. The high cumulative live-birth rates in this study can be explained by the effectiveness of the blastocyst vitrification technique.

Embryo cryopreservation has decreased the number of fresh ETs and maximized the effectiveness of the IVF cycle. However, there is still a major debate concerning the best stage, protocol, and cryoprotective additives to use. Cryopreservation of the remaining



IVF = *in vitro* fertilization.

Figure 1. A flow chart of the enrollment process of the patients. IVF = *in vitro* fertilization.

Table 1
Patient demographics and embryology data.

	Total population (n = 1137)
Age[a] (y)	32.7 ± 4.4
BMI[a] (kg/m^2)	22.6 ± 3.1
Duration of infertility[a] (y)	3.4 ± 2.6
Infertility factors	
Male factor, *n* (%)	407 (35.8)
Tubal factor, *n* (%)	229 (20.1)
Endometriosis, *n* (%)	180 (15.8)
PCOS, *n* (%)	129 (11.4)
Multiple factors, *n* (%)	183 (16.1)
Unexplained factor, *n* (%)	9 (0.8)
No. of retrieved oocytes[a]	19.9 ± 8.5
No. of fertilized oocytes[a]	13.3 ± 5.7
No. of blastocysts[a]	7.8 ± 3.5
No. of transferred blastocysts[a]	2.4 ± 0.7
No. of vitrified blastocysts[a]	4.0 ± 2.6

BMI = body mass index; PCOS = polycystic ovarian syndrome.
[a] The data are presented as mean ± standard deviation.

Table 2
Cumulative pregnancy rates after fresh and frozen embryo transfer.

	Fresh cycle (cycle 1)	1st frozen cycle (cycle 2)	2nd frozen cycle (cycle 3)
No. of ET cycles	1137	390	29
No. of pregnancies	747	250	17
Pregnancy rate[a] (%)	65.7	64.1	56.8
Cumulative pregnancy rate (%)	—	87.7	89.2
No. of multiple pregnancies	428	126	17
Multiple pregnancy rate (%)	37.6	32.3	58.6

ET = embryo transfer.
[a] There was no significant differences between the three groups.

Table 3
Cumulative live-birth rates after fresh and frozen embryo transfer.

	Fresh cycle (cycle 1)	1st frozen cycle (cycle 2)	2nd frozen cycle (cycle 3)
No. of ET cycles	1137	390	29
No. of live births	612	207	14
Live-birth rate[a] (%)	53.8	53.1	48.3
Cumulative delivery rate (%)	—	72.0	73.3

ET = embryo transfer.
[a] There was no significant differences between the three groups.

Table 4
Cumulative pregnancy rates of the different age groups after fresh and frozen embryo transfer.

Age (y)	No. of ET cycles		Fresh cycle (cycle 1)	1st frozen cycle (cycle 2)	2nd frozen cycle (cycle 3)	PR p[a]
<35	840	PR (%)	66.3 (557/840)	65.7 (186/283)	66.7 (10/15)	0.983
		CPR (%)	—	88.5 (743/840)	89.6 (753/840)	
35–39	278	PR (%)	64.0 (178/278)	60.0 (60/100)	50.0 (7/14)	0.478
		CPR (%)	—	85.6 (238/278)	88.1 (245/278)	
≥40	19	PR (%)	63.2 (12/19)	57.1 (4/7)	—	0.780
		CPR (%)	—	84.2 (16/19)	—	
CPR p				0.411		

CPR = cumulative pregnancy rate; ET = embryo transfer; PR = pregnancy rate.
[a] The p value is based on the Chi-square test.

Table 5
Cumulative live birth rates for different age groups after fresh and frozen embryo transfer.

Age (y)	No. of ET cycles		Fresh cycle (cycle 1)	1st frozen cycle (cycle 2)	2nd frozen cycle (cycle 3)	LBR p[a]
<35	840	LBR (%)	54.8 (460/840)	56.9 (161/283)	46.7 (7/15)	0.659
		CLBR (%)	—	73.9 (621/840)	74.8 (628/840)	
35–39	278	LBR (%)	51.8 (144/278)	43.0 (43/100)	50.0 (7/14)	0.320
		CLBR (%)	—	67.3 (187/278)	69.8 (194/278)	
≥40	19	LBR (%)	42.1 (8/19)	42.9 (3/7)	—	0.973
		CLBR (%)	—	57.9 (11/19)	—	
CLBR p				0.038		

CLBR = cumulative live-birth rate; ET = embryo transfer; LBR = live-birth rate.
[a] The p value is based on the Chi-square test.

supernumerary blastocysts after a fresh ET cycle is an option that should always be encouraged for potential use in future frozen cycles. A recent study shows that blastocyst transfer has a significantly higher cumulative pregnancy rate than a cleavage stage transfer in women 35 years or older [15]. Utilization of these blastocysts maximizes the cumulative pregnancy rate from a single IVF cycle [10,16].

There is no consensus on the superiority of any cryopreservation protocol. Two literature reviews and meta-analyses showed a higher post-thaw survival rate for vitrification, compared to slow freezing [17,18]. When evaluating the efficacy of vitrification versus slow cooling, it must be emphasized that embryo survival is an important endpoint, but is insufficient to determine which method is actually superior in clinical practice [19]. Therefore, the success of ART treatment should ultimately be defined by the live-birth rate when appraising the efficacy of vitrification versus slow freezing [19]. A recent population-based cohort study showed that vitrified blastocysts resulted in significantly higher live delivery rates, compared to slow-frozen blastocysts [20]. In our study, all remaining blastocysts were cryopreserved by vitrification.

The number of FET has increased in the past few years. The latest results generated from European registers by the European Society of Human Reproduction and Embryology demonstrate that embryo cryotransfers account for 21% of IVF activity. The pregnancy rate per thawing has increased since 2008 (19.3% in 2008, 20.9% in 2009, and 20.3% in 2010); this improvement may be related to the incorporation of vitrification in the embryology laboratory [21]. From 1997 to 2011 in the U.S., the number of FETs per initiated cycle has increased from 20% to 34% (i.e., a 70% increase) for women younger than 35 years [22]. The success rate after FET interestingly is nearly equal to that of fresh ET. In this study, the live-birth rate was 53.8% in the fresh cycles and 53.1% in the first vitrified cycles with no significant difference between the two cycles (Table 3). The result is consistent with the report from the U.S. CDC on ART success rates for all American fertility clinics in 2012 [4], which showed a similar live-birth rate between the fresh cycle and the FET cycle (46.9% vs. 42.0%, respectively).

Recent epidemiologic studies show an increased rate of adverse perinatal outcomes for children of fresh IVF cycles, compared with children of frozen ET cycles [23–26]. This increase does not occur in the donor oocyte population, which suggests that it is the periimplantation environment created after superovulation that is responsible for these changes [27]. Furthermore, some human data demonstrate greater receptive endometrium in cycles without ovarian stimulation, compared to cycles with stimulation [28–30]. Because of the aforementioned findings, some reports have hypothesized a so-called “freeze-all strategy” that may increase the success rates of IVF-ICSI treatment [5,22,27,31–35]. The outcomes of existing randomized trials appear to favor the strategy of frozen ET. However, high-quality randomized controlled trials should be performed to determine which cryopreservation protocol is best and whether a freeze-all strategy may completely abandon fresh transfers.

In general, the cumulative live-birth rate after IVF is reportedly between 45% and 55%. The quality and quantity of embryos are the two most important predictors of a completed IVF/ICSI cycle (i.e., fresh plus cryopreserved embryos transferred from one stimulated cycle) [36,37]. In addition, maternal age significantly reduces the cumulative live-birth rate [11]. A woman's age is a negative factor if she is 35–37 years old, and live-birth rates for IVF dramatically decrease beyond the age of 40 years [16,38].

Our cumulative live-birth rate (74.8%) was higher in females younger than 35 years, which is in agreement with previous publications [8,39]. In our study, the cumulative live-birth rates among women after two cycles (i.e., fresh cycle and the first frozen cycle) decrease with age (<35 years, 73.9%; 35–39 years, 67.3%; and ≥40 years, 57.9%), which was consistent with a previous report that demonstrated that live-birth rates are lower among older women than among younger women when autologous oocytes are used [10]. The low live-birth rate in women of advanced maternal age resulted from the high risk of producing aneuploid embryos. According to our previous unpublished preimplantation genetic screening data, the aneuploidy rate of embryos significantly increased from 42.5% for women younger than 35 years old to 72.5% for women 40 years or older.

Our improved cumulative live-birth rates could simply be a result of improved technology [40]. It is therefore difficult to compare our results with earlier studies. However, a major strength of this study is the use of a cohort of all women presenting for their first fresh IVF cycle, followed by a subsequent frozen transfer utilizing blastocysts of the same cohort. When the fresh cycle is unsuccessful, there is a higher likelihood that the embryos capable of generating a viable pregnancy in the cohort remain cryopreserved [41].

The limitation of this study is that the population of older women was relatively small. In this study, we only enrolled women younger than 42 years old who had undergone a blastocyst transfer; there were consequently only 19 older women (40–42 years old). Selection bias is therefore another limitation of this study.

In summary, this study showed high cumulative live-birth rates for women who underwent fresh and vitrified blastocyst transfer cycles and showed that the cumulative live-birth rates decrease with age. The success rates after FET is nearly equal to the success rates of fresh ETs. However, there is no clear choice that maximizes the success rates for all patients at all centers. Therefore, individualized approaches remain appropriate.

Conflict of interest

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

Acknowledgments

The authors received a research grant from Changhua Christian Hospital (grant number, 103-CCH-IRP-034).

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