The effect on pregnancy and multiples of transferring 1–3 embryos in women at least 40 years old

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Abstract

Purpose "Capsule" is mandatory. Please provide. Single embryo transfer (SET) in women ≥ 40 years old appears to lower the chance of a pregnancy. However, it minimizes the risk of multiple pregnancies even in women of advanced maternal age. Therefore, women 40 years of age or older should be offered (SET). This study was performed to investigate the multiple pregnancies and live birth rates when 1–3 embryos are transferred at this age in women at least 40 years of age. **Method** A retrospective analysis of data which included 631 women aged 40 to 46 years, who underwent 901 cycles of IVF, from August 2010 to June 2012 was undertaken. These women underwent embryo transfer of 1–3 non-donor fresh embryo(s).

Results Results suggested that the average pregnancy rate when up to three embryos were transferred was 25 % for women 40 years old, 20 % for women 41 years old, 16 % for women 42 years old, 17 % for women 43 years old, 8 % for women 44 years old, 6 % for women 45 years old, and 0 % for women 46 years old. No live births occurred in women treated after their 44th birthday, and only patients younger than 42 years of age receiving double embryo transfer had a live birth of twins. Live birth rates increased as more embryos were transferred for 40- and 42-year-old subjects ($p=0.01$ and 0.05 , respectively).

Capsule Further studies are needed to determine risk of multiple pregnancies in women 42 years of age or older when few embryos are transferred. Decisions on the number of embryos to transfer should be on a case by case basis, in discussion with the patient.

Conclusions From these results, it was concluded that SET in women ≥ 40 years old appears to lower the chance of a pregnancy. However, it minimizes the risk of multiple pregnancies even in women of advanced maternal age. Women 40 years of age or older should be offered single-embryo transfer. Further studies are needed to determine risk of multiple pregnancies in women 42 years of age or older when few embryos are transferred. Decisions on the number of embryos to transfer should be on a case by case basis, in discussion with the patient.

Keywords Assisted reproductive technology · Elective single-embryo transfer · Multiple pregnancy rates · Advanced maternal age

Introduction

The safest outcome of assisted reproduction for both the mother and the offspring is associated with a singleton pregnancy. However, the European Society of Human Reproduction and Embryology (ESHRE) reported that the twin pregnancy rate per embryo transfer in Europe was 19.2 % from in vitro fertilization (IVF) in 2011 [1]. While according to the American Society For Assisted Reproductive Technology (SART) data, the twin live birth rate was 23 % for all age groups, in 2014 [2]. It is usually in the age group of women 37 to 39 years where the highest multiple pregnancy rates occur after IVF [3]. This occurs since to encourage pregnancy in this age group, a greater number of embryos are transferred, yet embryo competence remains strong [3].

An extensive body of literature has demonstrated that twin gestations carry an increased risk of adverse outcomes, when compared to singletons. These risks include prematurity, low birth weight, higher infant mortality [4, 5], increased frequency of cerebral palsy, developmental delay, and maternal

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mortality [6]. Twin gestations also compound the health threats to women of advanced maternal age, who are already at increased risk of developing complications such as gestational diabetes [7] and preeclampsia [8]. Women over the age of 35 years with preeclampsia have a three-time higher risk of pregnancy-related mortality than do younger counterparts [9]. These adverse outcomes have a considerable impact on public health, leading to policies to decrease the prevalence of multiple gestations.

Given that the transfer of multiple embryos increases the risk of multiple gestations, many have suggested a move towards single-embryo transfer (SET). Randomized controlled trials have demonstrated that SET significantly diminishes the twin gestation rate and yields a comparable live birth rates in good prognosis patients [10, 11]. However, recommendations for SET have focused mainly on women less than 36 years of age [10]. Nevertheless, several studies have also addressed the role of SET in women between the ages of 36 and 39 years. One study found that SET could be applied to patients aged 36–39 years, achieving similar pregnancy rates (PRs) and a higher cumulative live birth rate compared to double embryo transfer (DET), while dramatically decreasing the multiple gestation rates (MGR) [12]. Another study found that SET could be offered to women younger than 38 years of age in the first three IVF treatment cycles without compromising the PR [13]. It should be noted that these studies have all been performed in good prognosis patients.

One study examined SET as compared to DET in women 40–43 years of age, in a European population [14]. This study found better pregnancy rates in the group which transferred a single embryo as compared to the DET group, although not statistically significant. This finding occurred even without considering the cumulative effect of subsequent frozen embryo transfers, which then heavily favored eSET. Given the findings in this single study, further research is needed, both because the results suggest undetected bias favoring pregnancy in the eSET group and due to the existence of significant differences in IVF success rates when comparing European and North American Clinics [15].

Blastocyst transfer (BT) has also emerged as a potential approach to reducing MGR as generally fewer embryos are transferred [16–18]. Array complete genome hybridization (a-CGH) is another technique, which enables the selection of genetically normal embryos, with high implantation potential [19]. However, most centers currently do not have access to a-CGH in their facilities, and it adds a considerable cost for the patients [20].

Many IVF centers in North America transfer 3 or more embryos in women older than 40 years old since the rate of aneuploidy is high and embryo competence is low [20]. An American cohort study estimated the MGR to be only 10.8 %, when 2 embryos were transferred in women 40 to 44 years of age [21]. In this age group, the likelihood of chromosomally

normal embryos are decreased compared to a younger population. The increased rate of aneuploidy in women 40 years of age or older is felt to lead to the majority of embryos arresting development [22]. Since most embryos in these women of advanced maternal age will not lead to a pregnancy, more embryos can be safely returned to the patient. However, Quebec on August 5, 2010 limited the number of embryos transferred to IVF patients to one in most cases. In certain cases, a maximum number of three embryos could be returned; however, justification was required to be documented in the medical chart. The aim of this law was to maintain the MGR under 10 %. This was done since the province was paying the cost of assisted reproductive technologies as well as care for the mother and offspring and any resultant complications through the provincial health-care plan. It was felt that limiting the cost of complications from multiple gestations could cover the cost of the ART funding. This mandate likely resulted in the fewest number of embryos transferred in women of advanced maternal age in North America. Limited data exists comparing SET with the DET and triple ET in North American women older than 40 years of age.

This study was performed to determine whether the PRs in this age group with SET differed significantly from those with a greater number of embryos transferred. It also investigated whether the multiple pregnancy rates are affected in light of high undetected aneuploidy rates in women 40 years old or older who had two or three embryos transferred, in which limited data is available.

Materials and methods

Data: This retrospective study analyzed the data of 631 women aged 40–46 years, who underwent a total of 901 cycles of IVF, with or without intracytoplasmic sperm injection (ICSI) treatment at the reproductive center over a 23-month period. The inclusion period of the study is from August 2010 till June 2012. This date range had a consequence that some live birth data became available in 2014. August 2010 was selected as a start date because this is when the Quebec government IVF program commenced (prior to this date, most women of this age group had four or more embryos returned). This program provides funded IVF for residents of the province for up to 3 cycles per live birth. This program also limits the number of embryo transfer in most cases to one; however, up to three embryos can be returned in selected cases.

The decision to transfer more than one embryo after implementing the Quebec law was based on clinical factors, including age, previous IVF cycles, current and previous treatment cycle response, number of previous embryos transferred, number of oocytes collected, and the number and quality of resultant embryos. Most importantly, the MGR must be maintained below 10 %. In early 2012, a decision was made at this

center to limit patients to the transfer of a maximum of two embryos, to control for a multiple pregnancy rate that was above the 10 % government mandate in the prior year.

Data on women 40 years and older who had one or more fresh embryo(s) transferred following oocytes retrieval were included. Women younger than 40 years of age, and women who received donor oocytes or embryos, were excluded. The database for each ART cycle performed at the reproductive center has been used as a data source for all parameters in this study, after obtaining approval from the Research Ethics Board.

For all subjects' age, treatment protocol, number of oocyte(s) collected, oocyte(s) maturity, number, stage and grade of embryo(s) transferred, PRs, clinical PR, MGRs, miscarriage rate, and live birth rate were obtained. Outcomes were stratified according to age groups.

In vitro fertilization: Ovarian stimulation was achieved by using human menopausal gonadotropins (hMG) (Ferring, Toronto, Canada) or recombinant follitropin alpha or beta (FSH) (Merck Serono, Mississauga, Canada) or (Merck Frost, Kirkland, Canada), with or without recombinant luteinizing hormone (r-LH) (Merck Serono, Mississauga, Canada). The initial dose of gonadotropins used was determined by the subjects' treating physicians according to the patient's age, the serum FSH level on day 3 of the cycle, and the antral follicle count at baseline ultrasound. The majority of our patients were treated with the microdose-flare protocol (488 cycles) [23] in which the gonadotropins were commenced 2 days after commencement of a low-dose gonadotropin releasing hormone (GnRH) agonist (Suprefact 50 mcg twice daily) (Sanofi-Aventis, Laval, Canada). Subsequently, the dosage of gonadotropins was adjusted according to the follicular response, as determined by serial ultrasound scans and serum estradiol levels.

The rest of the patients were treated with either a GnRH antagonist protocol (332 cycles) [24] or a GnRH agonist (GnRH-ag) long protocol (81 cycles) [25]. For the GnRH antagonist protocol, daily gonadotrophin injections were started on the second or the third day of either a spontaneous or an induced menstrual bleed. GnRH antagonist injections of Cetrotide (Merck Serono, Mississauga, Canada) or Orgalutran (Merck Frost, Kirkland, Canada) at a dose of 0.25 mg/day were started either on the sixth day of stimulation or when the leading follicle reached 14 mm. For the GnRH agonist long protocol, patients were pretreated with the 35-mcg ethanyl estradiol oral contraceptive pill (OCP). Daily subcutaneous GnRH-ag (Suprefact mcg/day) was started after 15–25 days or OCPs until inducing pituitary suppression and then reduced to (100–200 mcg/day) followed by gonadotropin stimulation.

Human chorionic gonadotropin (hCG) (Pharmaceutical Partners of Canada, Richmond Hill, Canada), or 250 mcg recombinant-hCG (choriogonadotropin alfa injection) (Merck Serono, Mississauga, Canada) was administered when the leading follicle mean diameter was ≥ 18 mm on transvaginal ultrasound. Ideally, hCG was injected when two follicles had

reached this diameter, although in subjects with a single follicle, this diameter was also used for triggering. Transvaginal oocyte recovery was performed 36–38 h after hCG administration, using a 17-gauge single-lumen or 16-gauge double-lumen cook aspiration needle (Cook, Adelaide, Australia).

After retrieval, oocytes were collected into COOK IVF medium (Cook, Adelaide, Australia) and inseminated with 10 μ L of prepared sperm, at a concentration of 5–7 million/mL of motile sperm, 38–41 h after hCG administration. In the case of ICSI, after retrieval, oocytes were collected similarly into COOK IVF medium. The oocytes were denuded of cumulus cells by using finely drawn glass pipettes after 1 min of exposure to 0.1 % hyaluronidase solution that was prepared for ICSI. Sperm samples for both IVF and ICSI were separated on three layer gradients of 95, 70, and 50 % PureSperm (Global, Boston, USA) by centrifugation at 500g for 20 min. Sperm pellets were washed twice in gamete buffer (Cook, Adelaide, Australia) by centrifugation at 200g for 10 min.

A single spermatozoon was injected into each metaphase II oocyte. After ICSI, each oocyte was transferred into a 20- μ L droplet of Cook cleavage medium (Cook, Adelaide, Australia) in a tissue culture dish under mineral oil. Fertilization was assessed 16–18 h after ICSI for the appearance of two polar bodies and two distinct pronuclei.

The embryo(s) transfer was performed under ultrasound guidance 2–5 days after oocyte retrieval, depending on the number and quality of the embryos available.

The luteal phase was supplemented with either micronized progesterone capsules (Merck-Frost, Kirkland, Canada) 200 mg vaginally, three times per day, progesterone gel 8 % (Merck Serono, Mississauga, Canada) vaginally two times a day, effervescent progesterone tablets (Ferring, Ontario, Canada) 100 mg vaginally three times per day, or intramuscular progesterone in oil (Cytex, Halifax, Canada) 50 mg per day; that was self-administered by each patient, starting from the day of oocyte collection up to the day of the pregnancy test and throughout the first trimester, if pregnant. The choice of progesterone type was left to the discretion of the treating physician.

Outcomes

A positive pregnancy test was defined as a serum β -hCG level of >10 IU/L, measured 16 days after oocyte retrieval. A clinical pregnancy was defined as a pregnancy with an intrauterine gestational sac at 6 weeks on transvaginal ultrasound (with or without fetal heart activity). A live birth was defined as a child born alive after 24 weeks gestational age.

Statistical analysis

The data were analyzed with Statistical Package for Social Sciences (SPSS 11.0, Chicago, IL). Continuous variables were assessed for a normal distribution using the Kolmogorov

Smirnov test. All variables were normally distributed. Continuous variables were analyzed with multivariate stepwise regression analysis to control for confounding effects and multiplicity. Variables used to control for confounding effects were treatment protocol, number of oocyte(s) collected, oocyte(s) maturity, number, stage and grade of embryo(s) transferred, day of embryo transfer, maternal age, paternal age, and number of previous IVF cycles. Categorical variables were analyzed using chi-squared tests or Fisher's exact tests. If in any cases cell numbers were 5 or less, corrections were applied as indicated.

Results

Data from 901 IVF cycles performed on 631 women at least 40 years old was collected. Two hundred and fourteen women had more than one IVF cycle. All women underwent ET with their own oocytes. They were divided according to their age at commence of treatment to 231 cycles performed on 40 years old women, 245 cycles performed on 41 years old women, 219 cycles performed on 42 years old women, 126 cycles performed on 43 years old women, 62 cycles performed on 44 years old women, 16 cycles performed on 45 years old women, and 2 cycles performed on 46 years old women.

The number of embryo(s) transferred as a function of age is presented in (Fig. 1). The number of embryo(s) transferred was statistically different at different ages ($p=0.005$). As can be noted from the figure, this is primarily due to an increased likelihood to transfer three embryos in women 42 and 43 years old than those who were 40 or 41 years old.

PRs were 25 % for women 40 years old, 20 % for women 41 years old, 16 % for women 42 years old, 17 % for women 43 years old, 8 % for women 44 years old, 6 % for women 45 years old, and 0 % for women 46 years old. When controlling for confounding effects, age was a significant predictor of pregnancy ($p=0.006$). No live births occurred in women treated after their 44th birthday. Therefore, only women below the age of 44 years, who underwent a total of 821 cycles, will continue to be discussed. The percentage of those patients who after having a positive pregnancy test continued to clinical pregnancies were as

follows: 84 % for the 40 years old, 69 % for the 41 years old, 71 % for the 42 years old, and 72 % for the 43 years old.

It should be highlighted (Table 1) that once patients are 42 years old, no twin deliveries were noted at this maternal age at conception or older, when using their own oocytes, although a greater percentage of women received a transfer of three embryos at 42 and 43 years of age.

As shown in (Table 2) compared to SET, the percentage of pregnancy in patients of all age groups increased up to 1.8 times higher when three embryos were transferred as compared to 1.8 and 1.4 times higher only in 40 to 42 years of age patients, respectively, when two embryos were transferred. Subsequently, as a result of SET, the singleton live birth rate (as a function of pregnancies) steadily decreased with advance age (54 to 20 %). This was the opposite of the trend seen as a steady increased use of triple embryo transfer (7 to 33 %) when comparing women from the age of 40 to 43 years at the time of care. This was expected given the likely lower implantation rates with advancing age.

As previously noted from (Table 1), only patients younger than 42 years of age and receiving only double embryo transfer had a live birth of twins. Single-embryo transfer did not result in any twin pregnancies. However, twin pregnancies did not occur once women were 42 years of age or greater even if more than one embryo was returned.

The effect of embryo quality on clinical outcomes was then evaluated. Since many subjects only had a single-embryo transfer, the quality of the best embryo was compared. Embryos are graded at McGill grade 1 (best) to grade 4 (worst). Embryo fragmentation rates are the following in: grade 1 10 %, grade 2 10–29.9 %, grade 3 30–50 %, and grade 4 >50 %. Grade 1 embryos are almost unheard of at this institution, and none were noted in this database.

Among women who had a day 2 embryo transfer, pregnancies ($p=0.03$) and clinical pregnancies ($p=0.03$) but not live births ($p=0.18$) were associated with higher grade best embryo transferred. When comparing the mean number of cells in the best embryo transferred, they did not differ for pregnancy ($p=0.68$) or clinical pregnancy ($p=0.22$). However, there were differences among those with a live birth (3.6 ± 0.6 vs.

Fig. 1 Probability of transferring one, two, or three embryos as a function of patient age

Table 1 Clinical pregnancy data

Age (years)	40 (%)	41 (%)	42 (%)	43 (%)
1 sacs	86	91	88	100
2 sacs	14	9	12	0
No fetal heart beat	12	17	13	31
1 fetal heart beat	75	71	74	69
2 fetal heart beats	12	11	13	0
Live birth(s) ^a	62	76	55	45
Singleton delivery ^a	85	90	100	100
Twin delivery ^a	15	10	0	0

Data presented as a percent of clinical pregnancies

^a Represented as a percent of live births

4.0±0.5 cells, $p=0.04$). This finding may suggest that embryos with more than four cells on day 2 do not favor live birth in women at least 40 years of age.

Among women who had a day 3 embryo transfer, pregnancies ($p=0.28$), clinical pregnancies ($p=0.82$), and live births ($p=0.40$) were not associated with higher grade best embryo transferred. When comparing the mean number of cells in the best embryo transferred, they did differ for pregnancy

($p=0.02$) but not for clinical pregnancy ($p=0.07$) or live birth ($p=0.13$).

Among women who had a day 4 embryo transfer, pregnancies ($p=1.0$), clinical pregnancies ($p=1.0$), and live births ($p=1.0$) were not associated with higher grade best embryo transferred. When comparing the mean number of cells in the best embryo transferred, they did not differ for pregnancy ($p=0.22$), clinical pregnancy ($p=0.27$), or live birth ($p=0.32$).

Patients who underwent more trials of IVF were much more likely to have more embryos transferred in subsequent cycles ($p=0.005$). As a result, the live birth rates of twins as a function of total live births were 3 % on the first trial of IVF, 25 % on the second transfer, and 15 % on the third embryo transfer. Mean ($\pm$ SD) number of embryos transferred on the first cycle was 1.9±0.68 embryos, second cycle 1.8±0.67 embryos, third cycle 2.0±0.72 embryos, and fourth cycle 2.1±0.75.

To further evaluate the roll that embryo quality played in multiple pregnancy rates, the data was subdivided based on blastocyst transfer. The results are presented in Table 3. As can be noted, the pregnancy rates, clinical pregnancy rates, and live birth rates as a function of embryo transfer were similar in

Table 2 Pregnancy outcome per number of embryo(s) transferred in different age groups

Age (years)	40	41	42	43	<i>p</i>
1 embryo transferred					
<i>N</i>	70	72	67	33	
Pregnancy rate	17 %	13 %	12 %	15 %	0.81
One sac	100 %	100 %	100 %	100 %	1.0
Two sacs	0 %	0 %	0 %	0 %	
Live birth singleton ^a	54 %	44 %	38 %	20 %	0.10
Live birth twins ^a	0 %	0 %	0 %	0 %	
2 embryos transferred					
<i>N</i>	132	135	104	55	
Pregnancy rate	28 %	23 %	17 %	15 %	0.11
One sac	79 %	86 %	85 %	100 %	0.63
Two sacs	21 %	9 %	15 %	0 %	
Live birth singleton ^a	40 %	35 %	11 %	25 %	0.79
Live birth twins ^a	11 %	10 %	0 %	0 %	
3 embryos transferred					
<i>N</i>	28	37	47	39	
Pregnancy rate	32 %	24 %	19 %	23 %	0.65
One sac	88 %	83 %	88 %	100 %	0.80
Two sacs	12 %	11 %	12 %	0 %	
Live birth singleton ^a	7 %	11 %	13 %	33 %	1.0
Live birth twins ^a	0 %	0 %	0 %	0 %	
<i>p</i> value associated with the number of embryo(s) transferred and pregnancy rate at each age group	0.37	0.36	0.51	0.52	
<i>p</i> value associated with the number of embryo(s) transferred and live birth rate at each age group (likelihood ratio)	0.02	0.63	0.05	0.16	

Sac(s) are represented as percent of subjects with one or two sacs

^a Represented as a percent of positive pregnancy tests

women with one or two blastocysts transferred. However, there was not enough data related to transfer of three blastocysts to draw any conclusions in this case. The likelihood of a twin birth trended greater ($p=0.06$), when two blastocysts were transferred when compared to one, without improving the likelihood of a pregnancy, clinical pregnancy, or live birth.

The contribution of the best blastocyst grade in women with and without a positive clinical outcome was then evaluated. Grade 1 blastocysts are almost unheard of at this institution, and none were noted in this database. The best blastocyst-transferred grade seemed to be trended better among women with a pregnancy ($p=0.08$), but not those with a clinical pregnancy ($p=0.43$) or live birth ($p=0.12$).

Discussion

It is increasingly evident in the developed world that women are delaying their attempts to conceive until their careers are stable [26]. This lead to a significant number of older age patients seeking assisted reproduction [27]. The Quebec government program for fertility care permits the evaluation of low-order embryo transfers on women ≥ 40 years of age for the first time. Single-embryo transfer has been proposed as a strategy to reduce the risk of multiple birth and adverse pregnancy outcomes after IVF. Multiple pregnancies are a problem at every age. However, as the pregnancy and live-birth rates fall with a concurrent rise in miscarriage and cycle cancellation rates in women older than 40 years old [28], the effect of multiple embryo transfer on MGR is poorly understood. Most centers transfer 4–5 embryos in women 40 years or older. An evaluation of transferring one to three embryos has not previously been evaluated in this age group.

Similar to other studies, our results failed to demonstrate any pregnancy in 46 years old women nor a live birth in the 80 cycles performed on women 44 years of age and older [29]. In another study on Bangladeshi women, the total

fecundability was constant until the age of 40 and then dropped rapidly to nearly zero by the age 46 [30]. It is well known that maternal age is the most significant prognostic factor for IVF success [31]. Various studies have consistently shown that oocytes from women over 40 years of age have reduced quality [31, 32]; subsequently, the resultant embryos will show decreased implantation and survival potentials [33].

Our data included the cycles started since the implementation of the Quebec law. Restricting the number of embryos transferred showed that even after transferring three embryos, no patient conceived triplets. In fact, it should also be pointed out that no patients 42 years old or older had a twin live birth, though the likelihood of transferring three embryos was high in this age group, as shown in Fig. 1. None of these patients underwent an elective reduction of twins. While 13 % of pregnant women at age of 42 years had two fetal heart beats, these all spontaneously reduced to singleton gestations. This would suggest that possible SET should be considered even in women at 42 years of age; however, further data will be needed to support this conclusion. No patients 43 years of age conceived more than a singleton pregnancy, irrelevant of the number of embryos transferred. Clearly, the data in this study suggests that it is primarily 40- and 41-year-old women who maintain an elevated risk of multiple gestations when using their own eggs. Given that women at these age groups are most at risk for complications due to twin gestations, clearly, SET should be offered to women 40 and 41 years of age, although it should be based on a case by case basis. It should be noted that pregnancy outcomes could not be determined based on embryo grade, in this age group. Although few women had poorest quality embryos transferred, these would likely have low implantation rates.

The overall MGR in our study was fairly low and is likely due to the low number of embryos transferred. Only single-embryo transfer can minimize the multiple pregnancy rates even in women at least 40 years of age. Although, our data suggests thought to transfer several

Table 3 A comparison of clinical outcomes as a function of number of blastocysts transferred ($N=127$)

Embryo quantity transferred	Pregnancy rate	Clinical pregnancy rate	Live birth rate
1 embryo ($N=38$)	45 %	34 %	21 %
2 embryos ($N=88$)	38 %	27 %	19 %
3 embryos ($N=1$)	0 %	0 %	0 %
p value from comparing 1 or more embryos transferred	0.54	0.60	0.82
	2 intrauterine sacs	2 fetal heart beats	Live birth twins
1 embryo	0 %	0 %	0 %
2 embryos	20 %	25 %	24 %
p value from comparing rate of multiples with 1 or 2 blastocysts transferred (likelihood ratio used)	0.06	0.05	0.06

Note: No triplets were noted. Two fetal heart beats and live birth twins represented as percent of pregnancies with live fetal heart beats and live births, respectively

embryos should be given to women 43 years of age. Clearly, a randomized trial in women 40 years or older would be elucidating. However, it is unlikely to occur due to the negative effects on pregnancy rates after transferring less embryos and the fact that in most countries patients are paying for their care. Therefore, the number of embryos to be transferred should be adjusted individually according to the patients' age, previous outcomes, and explanation of excellent pregnancy rates in frozen cycles with current technology.

An accent should be placed on the finding that transferring one or two blastocysts did not seem to change the pregnancy, clinical pregnancy, or live birth rate in women of this age. The grade of these blastocysts also did not affect the clinical pregnancy or live birth rates. In all cases, these blastocysts were grades 2 or 3. However, slightly higher rates of fragmentation, while remaining under 50 %, did not seem to affect the most important clinical outcomes. However, transferring two blastocysts likely increase the multiple rates as opposed to returning one ($p=0.06$ of twin live birth), without improving the live birth rate ($p=0.82$). Therefore, uterine transfer of one as opposed to two blastocysts in women at least 40 years of age is preferred.

Strengths of this study includes the large patient population and the limited ability of physicians' decisions to alter the number of embryos transferred, since it must follow strict government mandated guidelines irrelevant of embryo quality, the variety of stimulation protocols implemented, and the large number of physicians involved in the patient care (seven attending physicians). In addition, it is the first North American database to put back so few embryos in women 40 years of age or older. Weaknesses of this study include its retrospective nature.

Multiple pregnancies can still occur in women 40 years of age and older, when more than a single embryo is transferred. This rate is higher than the 10 % requirement provided in the Quebec government program for fertility care, and therefore, thought should be given to SET even in women 40 years of age and older. Clearly, single-blastocyst transfer should be used in women of advanced maternal age.

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