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Assisted Reproduction

Lower embryonic loss rates among twin gestations following assisted reproduction

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***Purpose*:** To determine whether maternal age and number of transferred embryos influence early pregnancy losses in twin pregnancies compared to singletons following IVF/ICSI.
***Methods*:** We compared the pregnancy loss rates in singleton ($n = 549$) and twin ($n = 252$) gestations, stratified by maternal age ($\leq$35 and >35 years) and the number of transferred embryos (1–3 and 4–9).
***Results*:** Loss rates of singleton pregnancies were significantly higher than that in twins (OR 3.0, 95% CI 1.9, 4.9), especially among singletons conceived after transfer of 4–9 embryos (OR 5.0, 95% CI 2.2, 11.9). Younger mothers of twins had lower loss rates (OR 0.3, 95% CI 0.1, 0.9).
***Conclusion*:** Twins have a significantly reduced spontaneous miscarriage rate compared with singletons following IVF/ICSI. Higher implantation rates per cycle (i.e., development of twins rather than one live embryo) may represent a better capacity of the uterus for early embryonic development.

KEY WORDS: ICSI; IVF; Pregnancy loss; Singletons; Twins.

INTRODUCTION

Early loss of the entire pregnancy, irrespective of plurality, is considered as miscarriage, whereas partial loss in a multiple pregnancy is usually considered in the context of spontaneous reduction (1) or the vanishing twin syndrome (2). Most texts on multiple gestations would submit to an increased embryonic loss among twin gestations compared to singletons. However, an intriguing finding of a recent comparative study indicates that pregnancy loss occurred in 5.1% of the in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) twin pregnancies compared to 21.1% in singletons (3). The authors maintained that the embryological potential for successful development is not the same for twins and singletons and that twin pregnancies after IVF/ICSI have a better survival potential than singletons. In another study, the frequency of spontaneous reduction after assisted reproduction was significantly lower in multiple gestations than in singletons (12.6 versus 20.8%) (4). Our recent study (5) fully supports these observations, and, in addition, shows that this is also the case for triplet and quadruplet gestations. One may argue that the increased loss of singletons in the abovementioned studies (3–5) represents an excessive loss rate reported from these centers only. However, similar loss rates of singletons following IVF were reported in other series (6,7).

Another explanation could attribute the advantage of multiples to the mode of conception, but the general observation, namely, a disadvantage for the early singleton pregnancies, was true for both ICSI and conventional IVF pregnancies (8). Finally, the data of two different studies show that the general

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trend does not consistently depend on maternal age (5,8).

A third explanation is that higher implantation rates per cycle (i.e., development of more than one live embryo) may represent a better uterine receptiveness for early embryonic development and/or better embryonic quality. Conversely, implantation of one embryo only, despite transfer of many embryos, may represent poor uterine receptiveness and/or poor embryonic quality, and hence, a higher miscarriage rate (5). This hypothesis indirectly corroborates that of Zegers-Hochschild *et al.* (4) who maintained that some women have a high reproductive efficacy. When these women are exposed to assisted reproductive technologies (ART), they produce better embryos, have a greater chance for more implantations, and consequently – they have a higher probability of having a multiple gestation. Following our initial studies (5,8), we conducted a secondary analysis to determine whether the number of transferred embryos in different maternal age groups influence these early spontaneous embryonic losses. The unique circumstance resulting from the ART protocols used in Reggio Emilia, where all available embryos are transferred in each cycle (5,8) enables this evaluation.

MATERIAL AND METHODS

During the study period January 1, 1992, to December 31, 2002, there were 1076 clinical pregnancies (positive heartbeat present on ultrasound examination) following ART in our non-private clinic at Arcispedale Santa Maria Nuova, at Reggio Emilia, Italy. This ART service has some unique features related to constraints prohibiting disposal, freezing of surplus embryos as well as multifetal pregnancy reduction. Moreover, only day 2–3 embryos are transferred without assisted hatching. We restricted our analysis to include 846 pregnancies following transfer of 1–9 embryos per cycle and to those resulting in either one or two live embryos visualized by first trimester sonography. We excluded cases were more than 9 embryos were transferred, or more than two live embryos were seen on sonography. The patients included in the study population comprised mainly (>95%) women of Italian-origin, who were all nulliparous, with a mean age of 34.2 ± 4.0 years. Data were prospectively collected in a standardized manner, regularly updated, and evaluated regularly for quality control of the collection process. Most patients received luteal support and those who did not were equally distributed among the respective study groups.

Following biochemical diagnosis of pregnancy, all pregnant patients underwent first-trimester transvaginal sonography at 4–5 weeks after ovum pick- up. The number of embryos with positive heartbeat in each pregnancy was counted and compared with the number of live embryos on a second trimester sonogram. This study focused on the loss rate of the entire pregnancy in cases that started as a singleton or as a twin gestation, stratified by maternal age ($\leq$35 and >35 years) and the number of transferred embryos (1–3 and 4–9). The cutoff value for the number of transferred embryos was arbitrarily chosen to differentiate between patients who had few or many transferable embryos.

The data were evaluated using the Microsoft Excel® software (Microsoft Corporation, Redmond, Washington). We used the True Epistat software (Math Archives, Round Rock, TX) to compare frequencies by the Fisher's exact tests. We derived odds ratio (OR) and Corenfield's 95% confidence intervals (CI). The ethical committee of the hospital approved the study. The same database was used for other studies (5,8).

RESULTS

The study groups comprised 594 and 252 pregnancies that started as singleton and twin pregnancies, respectively. Of the singleton pregnancies, 380 and 214 were in mothers aged $\leq$35 or >35 years, respectively; and 269 and 325 singletons followed 1–3 and 4–9 embryo transfers, respectively. Of the twin gestations, 169 and 83 pregnancies were in mothers aged $\leq$35 or >35 years, respectively; and 57 and 195 pregnancies followed 1–3 and 4–9 embryo transfers, respectively.

We examined the loss rates of entire pregnancies (Table I). The unadjusted loss rate of the entire singleton pregnancy was three times higher than that of twins. Transfer of 1–3 embryos did not affect the loss rate in both maternal age groups, whereas transfer of 4–9 embryos was associated with significantly fewer losses in pregnancies that started as twins. The effect of maternal age on loss rates was not apparent in singletons, but younger mothers had fewer losses of twins resulting from a larger number of transferred embryos.

Table I. Loss Rate of Entire Pregnancies Per First Trimester Live Pregnancies, by Maternal Age and Number of Transferred Embryos

Transferred embryos	Singletons	Twins	Total
≤ 35years			
1–3	39/182 (21.4)	3/36 (8.3)	42/218 (19.3)
4–9	48/198 (24.2)[c]	8/133 (6.0)[c,d]	56/331 (16.9)
Subtotal	87/380 (22.9)	11/169 (6.5)	98/549 (17.8)
>35 years			
1–3	25/87 (28.7)	4/21 (19.0)	29/108 (26.8)
4–9	37/127 (29.1)	10/62 (16.1)[d]	47/189 (24.9)
Subtotal	62/214 (29.0)	14/83 (16.9)	76/297 (25.6)
Total pregnancies	149/594 (25.1)[b]	25/252 (9.9)*b*	

[a]Data are shown as N (%).
[b]Odds ratio 3.0, 95% confidence interval 1.9, 4.9.
[c]Odds ratio 5.0, 95% confidence interval 2.2, 11.9.
[d]Odds ratio 0.3, 95% confidence interval 0.1, 0.9.

We also counted the rates of pregnancy losses in four groups, namely twins resulting from few (1–3) transferred embryos, singletons resulting from few transferred embryos, twins resulting from many (4–9) transferred embryos, and singletons resulting from many transferred embryos. The lowest loss rates were found among twin pregnancies resulting from either many or few transferred embryos (18/195, 9.2% vs. 7/57, 12.3%, respectively, not statistically different). The highest loss rates were found among singleton pregnancies resulting from either many or few transferred embryos (85/325, 26.1% vs. 64/269, 23.8%, respectively, not statistically different). However, the loss rates of twins were significantly different when compared to the loss rates of singletons (OR 3.1–3.5, 95% CI 1.7–2.0, 5.6–6.2). When maternal age was considered, the difference in loss rates between the four groups was seen only in mothers ≤35 years.

DISCUSSION

This paper focused on potential explanations for the relatively recent observation in pregnancies following IVF/ICSI, that twins carry a lower risk of embryonic loss compared with singletons (3–5). In a typical IVF program, few embryos are selected for transfer, and the surplus embryos are frozen, donated, or discarded. In contrast, within the special clinical constraints of the IVF service in Reggio Emilia, all transferable embryos are used. In this study, we did not evaluate the quality of the embryos nor the fertilization and implantation rate/embryo as proxies for embryo quality. In addition, we did not control the results for history of previous cycles. Thus, we are unable to comment on the specific relationship between embryo quality and the observed advantage for twins. However, differences in embryo quality seem unlikely to explain the different loss rates between first trimester singleton and twin gestations. This view is supported by the assumption that implantation is less likely with low-quality embryos (9), and would not make it to the stage of live embryos. This might explain the differences between our observation (which counted embryos with positive heartbeats) and studies that counted gestational sacs (1).

Another explanation may relate the mode of conception to the chance of embryonic loss. In the primary analysis of the same database, we found no difference between loss and/or survival rates of multiple gestations following conventional IVF or ICSI and suggested that other factors, such as embryo quality or uterine environment, might be involved in the outcome of multiple pregnancies following assisted reproduction (8).

It follows that a potential explanation should be sought within the uterine milieu of early pregnancy. Our data may suggest that higher implantation rates per cycle (i.e., development of twins rather than one live embryo following the transfer of 4–9 embryos) may represent a better capacity of the uterus for early embryonic development. This observation is highlighted by the fact that patients were similarly treated and not preselected for a given number of embryo transfers or pre-transfer embryonic quality (i.e., all day 2–3 appropriate embryos were transferred).

If the differences between lost gestational sacs and lost embryos are taken into account, the effect of twinning on embryonic survival does not seem to be an immediate post-implantation event. It can be speculated that early twin gestations may have a better utero-fetal interaction. A good candidate for this interaction is a superior hormonal support of the uterine milieu, produced by the well-recognized greater placental mass observed in early twin pregnancies. Some support to this speculation comes from the fact that twin placentas produce more hCG and progesterone than singleton placentas, and that both hormones are known to be associated with a reduction of the incidence of pregnancy loss (10–12).

Although our findings corroborate other observations in pregnancies following assisted reproduction (3,4), it is unknown whether they represent the situation in spontaneous conception. It is also unknown if the advantage of twins remains the same

in various regimens of hormonal support used in IVF protocols, or when transfers are performed at the blastocyst stage or of frozen-thawed embryos. Finally, it is unknown why some twin pregnancies are totally lost while others result in the "vanishing twin" syndrome. Nonetheless, our findings suggest that our intuitive view about the gloomy outcome of early twin gestations should be reappraised.

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