Journal of Assisted Reproduction and Genetics, Vol. 20, No. 8, August 2003 (© 2003)

Assisted Reproductive Technologies

Comparison of the Sex Ratio with Blastocyst Transfer and Cleavage Stage Transfer[1]

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Submitted January 14, 2003; accepted May 6, 2003

***Purpose*:** To evaluate the sex ratio in births conceived with blastocyst transfer compared to day 3-ET.
***Methods*:** A retrospective analysis of IVF patients who became pregnant after blastocyst or cleavage stage transfer at Stanford University Hospital and a literature review were performed.
***Result(s)*:** In the day 3-ET group, the male-to-female (M/F) ratio was 157/139 (53%/47%) compared to 97/66 (59.5%/40.5%) in the blastocyst group ($P = 0.18$). Similar trends have been found in individual studies in the literature but reached statistical significance in only one out of six reports reviewed. The combined data from our study and the literature show a male-to-female ratio of 797/594 (57.3%/42.7%) in blastocyst transfer compared to 977/932 (51.2%/48.8%) in day 3-ET ($P = 0.001$).
***Conclusion(s)*:** Although individual studies may lack power to show an altered sex ratio with blastocyst transfer, the combined data presented in this report do suggest that the M/F ratio is higher with blastocyst transfer compared to cleavage stage transfer.

KEY WORDS: Blastocyst transfer; cleavage stage transfer; IVF; sex ratio.

INTRODUCTION

Studies from several animal species have shown a higher male-to-female (M/F) sex ratio in the fastest developing embryos (1–4). Similarly, in human IVF, a greater likelihood of male offspring has been reported from cleavage stage embryos with higher cell numbers (5,6). By extending embryo culture to the blastocyst stage, it is likely that small or undetectable differences in cleavage rates on day 2 or 3 will become more obvious by day 5. Typically, IVF programs transfer the most expanded blastocysts and thus could be favoring conception with male embryos. In a multicenter study, Menezo *et al.* (7) reported a significantly higher M/F ratio in infants born after blastocyst transfer (BT) compared to those born after spontaneous conception in the same time period. A more relevant issue is to examine whether the M/F ratio is altered with BT compared to cleavage stage transfer. To answer this question, this study evaluates the sex ratio in births conceived with BT compared to day 3-ET.

MATERIALS AND METHODS

We retrospectively analyzed all births resulting from IVF cycles performed in our program over a 4-year period starting in January 1998 when BT was introduced to our center. The gender of the newborns was noted in both day 5 and day 3 transfer groups.

In addition, a literature search was performed to identify studies that specifically reported the sex ratio with BT, compared to day 3-ET. Abstracts from the American Society for Reproductive

[1] This paper was presented at the 58th Annual Meeting of the American Society for Reproductive Medicine, Seattle, Washington, October 2002.
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1058-0468/03/0800-0323/0 © 2003 Plenum Publishing Corporation

Medicine (ASRM) and European Society of Human Reproduction and Embryology (ESHRE) annual meetings since 1999 were reviewed as well.

Chi-square test was performed for statistical analysis and significance was set at $P < 0.05$. Institutional review board approval was obtained for chart review.

RESULTS

In the day 3-ET group, 237 deliveries resulted in 296 newborns with an M/F ratio of 157/139 (53%/47%). In the BT group, 132 deliveries resulted in 163 newborns with an M/F ratio of 97/66 (59.5%/40.5%). The difference between these ratios did not reach statistical significance ($P = 0.18$). There were four sets of monozygotic twins in the BT group and three sets in the day 3-ET group. Each of these incidences was counted as one newborn for the purpose of this study.

Six studies comparing the M/F ratio in BT and cleavage stage transfer were identified in the literature. Of those, two are published manuscripts (8,9). In one of these (9), the authors mentioned that the M/F ratio was not different for day 5 and day 3 transfers but did not provide the actual numbers. However, the data from their study was available in the proceedings of a national meeting (10). In addition, four abstracts that addressed this issue were found (11–14). Overall, only one of the six studies (14) showed a significant difference in the M/F ratio in BT (81/44) compared to day 3-ET (326/321), while the others demonstrated trends which did not achieve statistical significance (Table I). The combined data from our study and the literature show an M/F ratio of 797/594 (57.3%/42.7%) in BT compared to 977/932 in day 3-ET (51.2%/48.8%), which suggest that there is a significantly higher male occurrence with BT ($P = 0.001$).

DISCUSSION

Several published reports suggest that the fastest cleaving embryos tend to be more often male than female. In animal studies, the sex ratio of bovine IVF-derived blastocysts has been shown to be affected by their rate of development as reported by Avery *et al.* (1) The percentages of males after subdividing the embryos into three developmental groups were 68, 48, and 35% in the fast, intermediate, and slow groups, respectively ($P = 0.014$). Another study on IVF bovine cocultured blastocysts showed significantly more males, with the increase being more obvious with expanded blastocysts (2). IVF mouse embryos classified according to the time of morula to blastocyst transition showed 78, 57.5, and 42.5% males in fast, intermediate, and slow developing embryos, respectively (3). A study on pig in vivo conceptuses showed that at 10 days of pregnancy, males grew faster than females, with 17.6, 45.5, and 73% males for small, medium, and large embryos (4).

In human IVF, Pergament *et al.* (5) reported a significantly greater likelihood of liveborn males if the mean cell number per embryo was four or greater at the scheduled time of transfer. Tarin *et al.* (6) divided patients into negative, equal or positive selection groups depending on the difference between the mean number of cells in embryos transferred and all embryos available, being less than, equal to, or greater than zero. In singleton births, there were 90% males in the positive selection group compared to 89% females in the negative selection group. Similarly, Ray *et al.* (15) showed that male embryos cultured to the blastocyst stage had greater number of cells on day 2 than female embryos and that this difference was maintained up to the blastocyst stage in both the trophectoderm and inner cell mass. Conversely, Ng *et al.* (16) and Fanchin *et al.* (17) did not find any difference in the M/F ratio in relation to the cell numbers of the embryos transferred on day 2. However,

Table I. Sex Ratio with Blastocyst Transfer Compared to Day 3-ET

Study	M/F(%) day 3-ET	M/F(%) BT	Statistical significance
Meintjes *et al.* (11)	40/24 (62.5/37.5)	234/168 (58.2/41.8)	ns
Rodriguez *et al.* (12	49/51 (49/51)	47/35 (57.3/42.7)	ns
Anderson *et al.* (13)	108/95 (53.2/46.8)	83/67 (55.3/44.7)	ns
Mercader *et al.* (14)	326/321 (50.4/49.6)	81/44 (64.8/35.2)	s($P = 0.003$)
Wilson *et al.* (9,10)	128/134 (48.9/51.1)	163/143 (53.2/46.8)	ns
Kausche *et al.* (8)	169/168 (50.1/49.9)	92/71 (56.4/43.6)	ns
Present study	157/139 (53/47)	97/66 (59.5/40.5)	ns
Total	977/932 (51.2/48.8)	797/594 (57.3/42.7)	s($P = 0.001$)

it is possible that extended culture might allow subtle differences in embryo development on day 2 or 3 to become more apparent by day 5.

Since IVF programs tend to select blastocysts with good cellularity of the inner cell mass and trophectoderm, which also exhibit the greatest degree of expansion at the time of transfer, it is theoretically plausible that the sex ratio after blastocyst transfer will be skewed in favor of males. Although individual reports have typically shown trends for an increased M/F ratio with BT compared to day 3-ET, the small sample size per study has not allowed for statistical significance to be reached in most of these studies.

In a recent report, Kausche *et al.* (8) showed a sex ratio of 92/71 (56.4%/43.4%) with BT compared to 169/168 (50.1%/49.9%) with day 3-ET, which did not reach statistical significance. The authors felt that the lack of statistical significance in these results suggest that BT does not alter the sex ratio. Furthermore, they compared their findings to those of Menezo *et al.* (7), who reported an M/F ratio of 293/221 (57%/43%) which was significantly higher than that seen with spontaneous conception. Kausche *et al.* postulated that their practice to wait until day 6 in most of their blastocyst transfers, as opposed to Menezo *et al.*, who transferred embryos as soon as the blastocyst stage was reached, may account for the shift in sex ratio observed by Menezo *et al.* However, when the results from these two studies are compared, the difference in the M/F ratios of 92/71 (8) and 293/221 (7) is far from statistical significance ($P = 0.97$). Thus, it is quite possible that it is a matter of sample sizes more than anything else, which accounts for demonstrating or not demonstrating an alteration in the sex ratio with BT.

The data from our study, comparing the M/F ratio in BT and day 3-ET performed in the same program and same time period, show a trend for a shift in favor of males. When the findings from individual reports in the literature, including our own, are collectively examined, a significantly higher M/F ratio of 57/43 is seen with BT compared to 51/49 with cleavage stage transfer.

It is possible that the literature is biased by selective reporting. In other words, programs with equal numbers of males and females or higher numbers of females may not have reported their findings. However, since all of the studies with the exception of one (14) did not demonstrate a significant difference in the M/F ratio between BT and day 3-ET, it can be assumed that the authors' main objective was not to report that the M/F ratio is altered. As a matter of fact, trends for an increased M/F ratio with BT, which did not reach statistical significance, were dismissed by the authors who concluded that the sex ratio was not altered by BT. Thus, the conclusions of our study are not likely to be the results of biased reporting.

Ideally, the issue of sex ratio with BT compared to cleavage stage transfer should be assessed in a large prospective series. From a practical standpoint, however, the numbers required exceed what is available even in larger IVF programs. We realize the shortcomings of pooling data from multiple retrospective studies with potential variability in selection criteria and laboratory and clinical parameters. Unfortunately, the literature at present does not enable us to perform a well-designed meta-analysis. At this stage, we believe that our report offers valuable information about the likelihood of seeing a greater M/F ratio with blastocyst transfer.

CONCLUSIONS

Blastocyst transfer is certainly a valuable tool in the hands of IVF practitioners and has acquired acceptance by many programs throughout the world. In our own program, we have been strong proponents of BT and perform it routinely in cycles with multiple good quality-embryos (18–20). The ability of BT to reduce high-order multiple births through better embryo selection is a benefit of extended culture that, in our opinion, outweighs the finding of a slightly altered sex ratio as shown by our preliminary study. When weighing the advantages and disadvantages of extended culture, programs and patients should be aware of the possible increase in M/F ratio seen in day 5 transfers, but take into account that this phenomenon needs to be confirmed by a large, prospective randomized study.

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