SHORT COMMUNICATIONS

BUENOS AIRES, ARGENTINA

Deviation in Sex Ratio After Selective Transfer of the Most Developed Cocultured Blastocysts

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INTRODUCTION

In different mammalian species an early divergence in embryo growth rate related to sex has been described (1). Pergament *et al.* (2) studied the correlation between the average number of cells in embryos transferred on day 2 after insemination and the sex at birth of 36 singleton human pregnancies. They reported that more males were born when the mean number of cells per embryo was more than four at the time of transfer. Ray *et al.* (3) described an increase in number of cells and metabolic activity in male human preimplantation embryos.

In a series of human in vitro fertilization (IVF) procedures in which embryos were cocultured to the blastocyst stage before transfer, we observed a bias in the sex distribution that seems to be related to our habit of selecting the most developed embryos for transfer.

MATERIALS AND METHODS

Patients

Our study included 10 patients attending the assisted fertilization program at Halitus Instituto Médico, who were part of a group of 38 patients whose embryos were cocultured. All 10 women became pregnant after the transfer of embryos at the blastocyst stage. The mean age was 30.3 ± 2.8 years (range, 24 to 34 years).

IVF Procedure

Controlled ovarian hyperstimulation treatment was performed with the gonadotropin releasing hormone agonist (GnRHa) leuprolide acetate (Lupron, Abbot Argentina) starting at the midluteal phase of the previous cycle, follicle stimulating hormone (Metrodin; Serono Argentina), and human menopausal gonadotropin (Pergonal; Serono Argentina). When at least three follicles reached ≥ 18 mm, human chorionic gonadotropin (Profasi; Serono, Argentina) was injected, and after 36 hr oocytes were retrieved by ultrasound-guided transvaginal aspiration. In five cases fertilization was achieved by conventional IVF, and in the other five by intracytoplasmic sperm injection. HTF medium supplemented with 10% synthetic serum substitute (SSS; Irvine, Santa Ana, CA) was used for fertilization and embryo culture up to the four-cell stage. At that stage embryos were transferred to a confluent Vero cell monolayer. Cells and embryos were cocultured in α -minimum essential medium plus 10% SSS for 5 more days.

Embryo Transfer

On day 5, two or three embryos were transferred to the uterus with a Frydman catheter. Embryos were selected among those displaying more advanced development at the time of transfer, being at the expanded or full expanded blastocyst stage. Day 5 supernumerary blastocysts were cryopreserved, as well as blastocysts developing on days 6 and 7. In all cases sex was determined at delivery.

Statistical analysis of results was performed following the recommendations of Moore and Gledhill (4).

RESULTS

In the 10 pregnant patients included in this study, a total of 24 embryonic sacs was detected, 3 of which vanished before the end of the first trimester. Of those

Table I. Gender Distribution in Each Pregnant Patient

Patient	No. of fetuses	
	Male	Female
M.R.	2	1
C.S.	1	2
U.L.	2	0
R.W.	1	1
H.B.	1	1
P.S.	1	1
C.R.	1	0
C.S.	1	0
Z.D.	2	0
G.D.	3	0

21 that were born or are ongoing, 15 (71.4%) were identified as males and 6 (28.6%) were females. Considering the normal sex ratio of 0.517 for males and 0.483 for females, the results show a significant difference at the level of 0.035. Table I shows the sex distribution in the pregnancies. In all cases there was at least one male, and in half of the pregnancies only males were observed.

DISCUSSION

In studies performed by different authors on embryos from diverse mammalian species, asymmetry in the sex distribution at the blastocyst stage has been described (1). Pergament *et al.* (2) demonstrated that there is a trend toward faster development of male human embryos fertilized in vitro by day 2. Ray *et al.* (3) also found that the number of cells as well as the metabolic activity was greater in day 2 male embryos and that the difference was maintained up to the blastocyst stage.

Our results indicate that a significant degree of sex selection was involuntarily achieved after choosing the most developed blastocysts for transfer. Similar results were reported by other authors after transferring embryos at less advanced stages of development. The implantation rate of the blastocysts transferred to this group of patients was 100%, but unfortunately three sacs vanished during the first trimester. For that reason

it is not possible at present to say with certainty whether the observed bias in the sex ratio is due to human male embryos developing faster than female ones grown under the same conditions or if it was caused by some kind of maternal effect, since all the patients considered herein belong to the age group indicated by Tarin *et al.* (5) to be shifted to male selection. In this small group of patients in which two or three blastocysts were transferred per woman, all pregnancies were multiple. For that reason, now our policy is to transfer only one expanded blastocyst when performing coculture.

We speculate that after performing single embryo transfers, and paying special attention to morphology and time of transfer, a better relationship between embryo developmental stage and sex would probably be observed. Study of future pregnancies would be helpful as a confirmation of the conclusions drawn here and would also clarify whether this finding has any practical application.

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