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Sex selection may be inadvertently performed in in-vitro fertilization–embryo transfer programmes

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The present study aims to ascertain whether sex selection may be inadvertently performed in human in-vitro fertilization (IVF) and embryo transfer (IVF–embryo transfer) programmes when selecting for high quality embryos (those with the fastest cleaving rates and/or the best morphology) at the fresh transfer cycle. All patients entering into the study were treated with gonadotrophins after pituitary suppression with gonadotrophin-releasing hormone agonists (GnRHa) and had intrauterine embryo transfer on day 2 post-insemination. These patients were retrospectively divided into three groups according to whether the difference in mean number of cells between embryos transferred and all embryos available for transfer in a given cycle was less than (negative selection), equal to (no selection) or greater (positive selection) than zero. In cycles resulting in singleton births, the sex ratio of the resulting babies was significantly ($P \leq 0.005$) shifted toward the female (88.8%) and to the male (90.0%) in the negative and positive selection groups respectively. No shift in sex ratio was observed in cycles resulting in multiple births. Maternal age was another independent factor affecting sex ratio at birth. Sex ratio was significantly ($P \leq 0.05$) skewed in favour of males (62.7%) and females (71.4%) in women <35 and ≥35 years of age respectively. Maternal age, number of embryos transferred and the event of selecting or not selecting the slowest cleaving embryos for transfer were entered automatically in a three-group discriminant model for distinguishing cycles resulting in only boys, both boys and girls, and only girls. These data suggest that (i) sex selection may be inadvertently performed in IVF–embryo transfer programmes when selecting for high quality embryos at the fresh transfer cycles; (ii) human endometria may be favourable, indifferent or hostile to either fast cleaving or slow cleaving embryos depending on maternal age; and (iii) 'natural' sex selection may be performed for social, psychological or medical reasons.

Key words: embryo cleavage rate/in-vitro fertilization/maternal age/pregnancy outcome/sex selection

Introduction

Since Trounson and Mohr reported in 1983 the first human pregnancy following cryopreservation, thawing, and transfer of an 8-cell embryo, this technique has become a valuable adjunct to most human in-vitro fertilization (IVF) and embryo transfer (IVF–embryo transfer) programmes around the world. The introduction of this technique has given rise, however, to different philosophies or strategies for choosing the embryos to be transferred at the fresh cycle. Basically, IVF–embryo transfer programmes with cryopreservation facilities either select for high quality embryos (those with the fastest cleaving rates and/or the best morphology) at the fresh transfer cycle or do not carry out any embryo selection at all (Garrisi and Navot, 1992). IVF–embryo transfer programmes with no cryopreservation facilities select for the highest quality embryos at the fresh transfer cycle and the surplus embryos are donated to synchronous recipients, used for research purposes or simply discarded.

Although there are exceptions (King *et al.*, 1991; Berg *et al.*, 1992; Grisart *et al.*, 1995), it has been shown by many authors that mammalian male embryos during the post-implantation period (Scott and Holson, 1977; Pedersen, 1980; Seller and Perkins-Cole, 1987), as well as during the pre-implantation period *in vivo* (Avery *et al.*, 1989; Burgoyne, 1993; Cassar *et al.*, 1994) or *in vitro* (Tsunoda *et al.*, 1985; Avery *et al.*, 1991, 1992; Xu *et al.*, 1992; Valdivia *et al.*, 1993; Yadav *et al.*, 1993; Zwingman *et al.*, 1993; Pergament *et al.*, 1994; Peippo and Bredbacka, 1995) cleave and/or develop faster than female embryos. Hence, the philosophy of selecting for the highest quality embryos at the fresh transfer cycle in IVF–embryo transfer programmes may discriminate against female embryos and generate a bias on sex ratio of the resulting babies.

The present study aims to verify whether sex selection may indeed be inadvertently performed in IVF–embryo transfer programmes. If so, one may ask whether 'natural' sex selection may be used at whim or for preventing feticide, infanticide and homicide of females; X-linked (male) disorders; or parental psychological distress.

Materials and methods

The data from the present study are from infertile IVF embryo transfer couples who had had intrauterine embryo transfer at the Hospital Clínico Universitario de Valencia and Instituto de Fertilidad y Ginecología de Alicante between 1988 and 1994. All women entering into the study underwent pituitary desensitization by s.c. administration of gonadotrophin-releasing hormone agonists (GnRHa) before starting ovarian stimulation with a combination of follicle stimulating hormone (FSH) and human menopausal gonadotrophin (HMG).

Table I. Epidemiological variables in in-vitro fertilization (IVF) embryo transfer cycles resulting in either singleton or multiple births according to whether embryos were or were not selected for transfer

	Singleton births			Multiple births		
	Negative selection[a]	No selection[b]	Positive selection[c]	Negative	No selection	Positive selection
No. of cycles	9	20	10	8	8	5
Maternal age[d] (years)	31.8 ± 1.1	32.9 ± 0.8	30.9 ± 1.3	30.8 ± 1.4	29.0 ± 0.8	31.2 ± 2.2
Cause of infertility						
Tube peritoneal	6 (66.7)	14 (70.0)	6 (60.0)	7 (87.5)	5 (62.5)	4 (80.0)
Unexplained	2 (22.2)	2 (10.0)	1 (10.0)	–		–
Male	1 (11.1)	3 (15.0)	3 (30.0)	1 (12.5)	2 (25.0)	1 (20.0)
Endometriosis	–	1 (5.0)		–	1 (12.5)	–

Values in parentheses are percentages.
[a,b,c]Difference in mean number of cells between embryos transferred and all embryos available for transfer [a]less, [b]equal or [c]greater than zero.
[d]Values are means ± SE.

Oocyte corona–cumulus complexes were obtained by ultrasound guided transvaginal follicular aspiration. Spermatozoa for insemination were selected by swim-up. Normally fertilized embryos (presence of two pronuclei and two polar bodies 17–19 h after insemination) were transferred to the uterus 42–44 h after insemination. If available, a maximum of three to four high-quality embryos per patient was transferred and the remaining embryos were cryopreserved. Although some frozen–thawed embryos were transferred in later cycles, only the transfer of fresh embryos during the IVF–embryo transfer cycle has been considered for the purposes of this study.

In order to verify whether sex selection may be inadvertently performed by choosing the fastest cleaving embryos at the fresh transfer (or alternatively by choosing the slowest cleaving embryos in cases in which slow-cleaving embryos exhibited better morphology than fast-cleaving embryos), the mean number of cells at the time of fresh transfer was recorded. Three groups were established according to whether the difference in mean number of cells between embryos transferred and all embryos available for transfer in a particular cycle was less than (negative selection), equal to (no selection) or greater (positive selection) than zero.

For mean comparisons among and between groups, analysis of variance (ANOVA) and Student's *t*-test were applied respectively. Proportions were transformed to arcsine before carrying out mean comparisons. When ANOVA test showed statistical differences, Student Newman–Keuls (SNK) test was used to discriminate between groups. χ^2 test and Fisher's exact test, if any expected cell value in a 2×2 table was <5, were applied for comparisons of frequencies. Three-group discriminant analysis was applied for finding predictor variables capable of distinguishing among IVF–embryo transfer cycles resulting in only boys, both boys and girls, and only girls. In this analysis, automated stepwise variable selection based on minimization of Wilks' statistic was used. Equality of group covariance matrices was tested by applying Box's *M* test. Significance was defined as $P \leq 0.05$. The entire statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS).

Results

Tables I, II, and III show epidemiological, ovarian stimulation, and IVF–embryo transfer variables from cycles resulting in either singleton or multiple births after selecting or not selecting the embryos for transfer. No significant differences in maternal age and distribution of causes of infertility (Table I) and ovarian stimulation variables (Table II) among groups were found. In cycles resulting in singleton births, the number of oocytes retrieved was significantly ($P \leq 0.05$) reduced in the non-selection group when compared to the negative and the positive selection groups (Table III). The mean number of cells of embryos transferred was significantly ($P \leq 0.05$) reduced in the non-selection group when compared to the positive selection group. Differences in sex distribution were highly significant ($P \leq 0.005$) among groups. An inversion of the male to female ratio was observed in cycles in which the slowest cleaving embryos were chosen for transfer (0.1, negative selection group) when compared to cycles in which the fastest cleaving embryos were transferred (9.0, positive selection group). This inversion of sex ratio was not observed in cycles resulting in multiple births. In these cycles, the mean number of embryos transferred was significantly ($P \leq 0.05$) lower in the negative selection group when compared to the non-selection and the positive selection groups.

Table IV shows the sex distribution of both singleton and multiple births according to whether mean number of cells of embryos transferred was less than, equal to or greater than their respective medians (3.5 and 3.4 for cycles resulting in singleton and multiple births respectively). No significant differences in sex distribution between groups were observed.

In order to search for further variables that may affect sex ratio of IVF children at birth, epidemiological, ovarian stimulation, and IVF–embryo transfer variables were compared among cycles resulting in only boys, both boys and girls or only girls. No significant differences in distribution of causes of infertility and ovarian stimulation variables among groups were found. However, women giving birth to only girls were significantly ($P \leq 0.05$) older (33.5 ± 0.7 years) than women giving birth to only boys (31.1 ± 0.7 years) or women giving birth to both boys and girls (29.3 ± 0.8 years). Mean number of embryos transferred ($P \leq 0.05$) and implantation rate per embryo transferred ($P \leq 0.01$) were significantly higher in cycles resulting in both boys and girls (Table V). The distribution of singleton and multiple births was significantly ($P \leq 0.0001$) different among groups.

The effect of maternal age on sex ratio was analysed to ascertain whether there was an interaction between this variable and type of embryo selection performed for transfer (negative, null, or positive). No significant differences in distribution of causes of infertility and ovarian stimulation variables were

Table II. Ovarian stimulation in in-vitro fertilization (IVF)–embryo transfer cycles resulting in either singleton or multiple births according to whether embryos were or were not selected for transfer

	Singleton births			Multiple births		
	Negative selection	No selection	Positive selection	Negative	No selection	Positive selection
Duration of GnRHa treatment before ovarian stimulation (no. of days)	13.8 ± 2.1	15.2 ± 1.6	18.7 ± 2.9	16.0 ± 1.5	14.3 ± 1.9	12.0 ± 1.7
FSH (no. of ampoules)	14.2 ± 4.5	11.1 ± 2.2	12.7 ± 3.8	10.0 ± 0.0	7.5 ± 1.1	9.4 ± 4.1
HMG (no. of ampoules)	35.9 ± 6.1	28.7 ± 2.3	24.1 ± 0.8	22.3 ± 2.9	24.5 ± 3.7	30.0 ± 1.8
FSH + HMG (no. of ampoules)	43.6 ± 5.9	35.3 ± 2.3	31.4 ± 1.6	32.3 ± 2.9	32.0 ± 3.0	35.5 ± 2.8
Duration of ovarian stimulation (no. of days)	12.4 ± 0.7	11.2 ± 0.5	10.3 ± 0.5	9.6 ± 0.6	10.6 ± 0.9	11.6 ± 0.8
Serum oestradiol (pg/ml) on day of HCG administration	2041.9 ± 397.6	1379.7 ± 142.1	2068.1 ± 389.4	1973.6 ± 305.9	1928.8 ± 329.1	1965.0 ± 545.8

Values are mean ± SE.
GnRHa = gonadotrophin-releasing hormone agonist; FSH = follicle stimulating hormone; HMG human menopausal gonadotrophin.

Table III. In-vitro fertilization (IVF)–embryo transfer variables in cycles resulting in either singleton or multiple births according to whether embryos were or were not selected for transfer

	Singleton births			Multiple births		
	Negative selection	No selection	Positive selection	Negative	No selection	Positive selection
No. of oocytes retrieved	13.3 ± 2.3	6.4 ± 0.8[a]	13.5 ± 2.7	14.3 ± 1.1	11.6 ± 3.2	13.8 ± 1.6
Fertilization (%)	72.1 ± 5.4	69.6 ± 5.7	66.9 ± 7.6	75.5 ± 3.4	59.2 ± 9.5	71.2 ± 6.0
No. of cells of all embryos available for transfer	3.6 ± 0.2	3.3 ± 0.2	3.7 ± 0.3	4.1 ± 0.3	3.6 ± 0.2	2.9 ± 0.4
No. of cells of embryos transferred	3.2 ± 0.2	3.3 ± 0.2[b]	4.1 ± 0.3	3.7 ± 0.3	3.6 ± 0.2	3.5 ± 0.4
Embryos transferred	3.0 ± 0.2	3.2 ± 0.2	3.3 ± 0.2	3.3 ± 0.2[c]	4.1 ± 0.2	4.0 ± 0.3
Implantation per embryo transferred	13/27 (48.1)	23/64 (35.9)	12/33 (36.4)	19/26 (73.1)	24/33 (72.7)	11/20 (55.0)
Fetal wastage						
Gestational sacs re-absorbed	4 (30.8)	3 (13.0)	2 (16.7)	1 (5.3)	3 (12.5)	–
Births						
Boys	1 (11.1)[d]	12 (60.0)	9 (90.0)	11 (61.1)	13 (61.9)	5 (45.5)
Girls	8 (88.8)	8 (40.0)	1 (10.0)	7 (38.9)	8 (38.8)	6 (54.5)

Values are means ± SE; values in parentheses are percentages.
[a]Value significantly different from the negative selection and the positive selection groups ($P \leq 0.05$).
[b]Value significantly different from the positive selection group ($P \leq 0.05$).
[c]Value significantly different from the non-selection and the positive selection groups ($P \leq 0.05$).
[d]Sex distribution significantly different among groups under singleton births ($P \leq 0.005$).

Table IV. Sex distribution in in-vitro fertilization (IVF)–embryo transfer cycles resulting in either singleton or multiple births according to whether the number of cells of embryos transferred was less than, equal to or greater than their respective medians

	Singleton births		Multiple births	
	No. of cells ≤3.5	No. of cells >3.5	No. of cells ≤3.4	No. of cells >3.4
Births				
Boys	9 (47.4)	13 (65.0)	15 (53.6)	14 (63.6)
Girls	10 (52.6)	7 (35.0)	13 (46.4)	8 (36.4)

Percentages are shown in parentheses.

found between women aged < 35 and those aged ≥ 35 years. Mean number of cells of all embryos available for transfer ($P \leq 0.005$), mean number of cells of embryos transferred ($P \leq 0.005$), and mean number of embryos transferred ($P \leq 0.05$) were all significantly higher in women <35 years of age (Table VI). The distribution of type of embryo selection performed for transfer was not significantly different between groups. However, a significant ($P \leq 0.05$) inversion of male to female ratio was observed in women ≥35 years of age (0.4) when compared to women <35 years of age (1.7).

Three-group discriminant analysis was applied in order to evaluate whether maternal age, mean number of cells of embryos transferred, mean number of embryos transferred, and three dichotomous variables resulting from selecting or not selecting the slowest cleaving embryos, the fastest cleaving embryos, or the average-cleaving embryos for transfer, were important variables for distinguishing among cycles resulting in only boys, both boys and girls, and only girls. Only maternal age, mean number of embryos transferred, and the event of selecting or not selecting the slowest cleaving embryos for transfer were entered into the discriminant model. Box's *M* test was non-significant ($P = 0.7582$) and therefore we had no grounds to reject the null hypothesis that the group

Table V. In-vitro fertilization (IVF) embryo transfer variables according to sex outcome at birth

	Only boys	Both boys and girls	Only girls
No. of cycles	27	14	19
No. of oocytes retrieved	10.1 ± 1.3	13.1 ± 1.9	10.7 ± 1.4
Fertilization (%)	69.3 ± 4.2	68.8 ± 6.0	68.9 ± 4.9
No. of cells of all embryos available for transfer	3.5 ± 0.1	3.6 ± 0.2	3.5 ± 0.2
Embryo selection for transfer			
Negative	4 (14.8)	4 (28.6)	9 (47.4)
No selection	13 (48.1)	7 (50.0)	8 (42.1)
Positive	10 (37.0)	3 (21.4)	2 (10.5)
No. of cells of embryos transferred	3.6 ± 0.2	3.6 ± 0.2	3.3 ± 0.2
Embryos transferred	3.3 ± 0.2	4.0 ± 0.2[a]	3.1 ± 0.2
Implantation rate per embryo transferred	37/88 (42.0)[b]	38/56 (67.9)	27/59 (45.8)
Multiplicity of pregnancy			
Singleton	22 (81.5)[c]		17 (89.5)
Multiple	5 (18.5)	14 (100.0)	2 (10.5)
Fetal wastage			
Gestational sacs re-absorbed	4 (10.8)	4 (10.5)	5 (18.5)

Values are means ± SE; percentages are shown in parentheses.
[a]Value significantly different from IVF–embryo transfer cycles resulting in only boys and only girls ($P \leq 0.05$).
[b,c]Distribution significantly different among groups ([b]$P \leq 0.01$; [c]$P \leq 0.0001$).

Table VI. In-vitro fertilization (IVF)–embryo transfer variables in cycles resulting in at-term pregnancy according to maternal age (years)

	<35 years	≥35 years
No. of cycles	48	12
No. of oocytes retrieved	11.4 ± 1.0	9.3 ± 1.7
Fertilization (%)	70.2 ± 3.0	64.6 ± 7.0
No. of cells of all embryos available for transfer	3.7 ± 0.1[a]	2.9 ± 0.3
Embryo selection for transfer		
Negative	14 (29.2)	3 (25.0)
No selection	22 (45.8)	6 (50.0)
Positive	12 (25.0)	3 (25.0)
No. of cells of embryos transferred	3.7 ± 0.1[a]	2.9 ± 0.2
No. of embryos transferred	3.5 ± 0.1[b]	2.9 ± 0.2
Implantation rate per embryo transferred	86/168 (51.2)	16/35 (45.7)
Multiplicity of pregnancy		
Singleton	29 (60.4)	10 (83.3)
Multiple	19 (39.6)	2 (16.7)
Fetal wastage		
Gestational sacs re-absorbed	11 (12.8)	2 (12.5)
Births		
Boys	47 (62.7)[c]	4 (28.6)
Girls	28 (37.3)	10 (71.4)

Values are means ± SE; percentages are shown in parentheses.
[a,b]Value significantly different from women ≥35 years of age ([a]$P \leq 0.005$; [b]$P \leq 0.05$).
[c]Sex distribution significantly different between groups ($P \leq 0.05$).

covariance matrices were equal. Table VII shows the unstandardized canonical discriminant function coefficients and the percentages of correct (they appear on the diagonal of the table since the predicted and the actual groups are the same) and incorrect classifications. The misclassification rates were 51.9% (14/27), 21.4% (3/14), and 21.1% (4/19) in cycles resulting in only boys, both boys and girls, and only girls respectively.

Table VII. Numbers of correct and incorrect classifications of sex outcome at birth according to the discriminant model shown below[a]

Actual group	Predicted group membership		
	Only boys	Both boys and girls	Only girls
Only boys	13/27 (48.1)	7/27 (25.9)	7/27 (25.9)
Both boys and girls	1/14 (7.1)	11/14 (78.6)	2/14 (14.3)
Only girls	2/19 (10.5)	2/19 (10.5)	15/19 (78.9)

Percentages are shown in parentheses.
[a]Unstandardized canonical discriminant function coefficients:

	Function 1	Function 2
Transfer or not of slowest cleaving embryos	0.9885902	1.8341000
Maternal age	0.2278918	0.0851418
Number of embryos transferred	–0.3830681	1.0839860
Constant	6.1474540	–6.8634370

Table VIII. In-vitro fertilization (IVF)–embryo transfer variables in cycles resulting or not in pregnancy according to whether embryos were or were not selected for transfer

	Negative selection	No selection	Positive selection
No. of cycles	63	225	66
No. of oocytes retrieved	13.5 ± 0.9	8.0 ± 0.3[a]	13.6 ± 0.9
Fertilization (%)	65.8 ± 2.5	55.1 ± 1.9[a]	69.2 ± 2.4
No. of cells of all embryos available for transfer	3.7 ± 0.1[b]	3.4 ± 0.1	3.3 ± 0.1
No. of cells of embryos transferred	3.2 ± 0.1	3.4 ± 0.1	3.9 ± 0.1[c]
Embryos transferred	3.3 ± 0.1	2.8 ± 0.1[a]	3.5 ± 0.1
Implantation rate per embryo transferred	32/207 (15.5)	60/640 (9.4)	26/228 (11.4)
Pregnancy rate per embryo transfer	17 (27.0)	39 (17.3)	18 (27.3)
Multiplicity of pregnancy			
Singleton	5 (29.4)[d]	27 (69.2)	11 (61.1)
Multiple	12 (70.6)	12 (30.8)	7 (38.9)
Fetal wastage			
Gestational sacs re-absorbed	5 (15.6)	9 (15.0)	2 (7.7)
Fetuses miscarried	–	10 (16.7)	3 (11.5)

Values are means ± SE; percentages are shown in parentheses.
[a]Value significantly different from the negative selection and the positive selection groups ($P \leq 0.05$).
[b]Value significantly different from the non-selection group ($P \leq 0.05$).
[c]Value significantly different from the negative selection and the non-selection groups ($P \leq 0.05$).
[d]Distribution significantly different among groups ($P \leq 0.05$).

In order to test whether the probability of both implantation and pregnancy rates were diminished in those cycles in which the slowest cleaving embryos were selected for transfer, data were divided into three groups according to whether embryos were or were not selected for transfer. On this occasion, all transfer cycles whether resulting or not in pregnancy were entered into the analysis. No significant differences in maternal age, distribution of causes of infertility, and ovarian stimulation variables among groups were found. However, the concentrations of serum oestradiol on day of human chorionic gonadotrophin (HCG) administration were significantly ($P \leq 0.05$) lower

Table IX. Pregnancy outcome according to whether embryos were or were not selected for transfer and women's age (years)

	Negative selection		No selection		Positive selection	
	<35 years	≥35 years	<35 years	≥35 years	<35 years	≥35 years
No. of cycles	49	14	146	79	44	22
Implantation per embryo transferred	28/163 (17.2)	4/44 (9.1)	47/422 (11.1)[a]	13/218 (6.0)	20/158 (12.7)	6/70 (8.6)
Pregnancy rate per embryo transfer	14 (28.6)	3 (21.4)	27 (18.5)	12 (15.2)	14 (31.8)	4 (18.2)
Multiplicity of pregnancy						
Singleton	3 (21.4)	2 (66.7)	16 (59.3)	11 (91.7)	9 (64.3)	2 (50.0)
Multiple	11 (78.6)	1 (33.3)	11 (42.3)	1 (9.1)	5 (35.7)	2 (50.0)
Fetal wastage from singleton pregnancies						
Gestational sacs re-absorbed		–	–	1/11 (9.1)		–
Fetuses miscarried	–	–	4/16 (25.0)	5/11 (45.5)	2/9 (22.2)	1/2 (50.0)
Fetal wastage from multiple pregnancies						
Gestational sacs re-absorbed	5/25 (20.0)	–	7/31 (22.6)	1/2 (50.0)	1/11 (9.1)	1/4 (25.0)
Fetuses miscarried	–		1/31 (3.2)	–	–	
Births from singleton pregnancies						
Boys	–		10 (83.3)	1 (25.0)	6 (85.7)	1 (100.0)
Girls	3 (100.0)	2 (100.0)	2 (16.7)	4 (75.0)	1 (14.3)	–
Births from multiple pregnancies						
Boys	11 (55.0)	1 (50.0)	14 (60.9)	–	6 (60.0)	1 (33.3)
Girls	9 (45.0)	1 (50.0)	9 (39.1)	1 (100.0)	4 (40.0)	2 (66.7)

Percentages are shown in parentheses.
[a]Value significantly different from women ≥35 years of age ($P \leq 0.05$).

in the non-selection group (1702.7 ± 61.0 IU/ml) when compared to the negative selection (2060.2 ± 125.6 IU/ml) and the positive selection (2224.8 ± 119.5 IU/ml) groups. The non-selection group also showed a significantly ($P \leq 0.05$) decreased number of oocytes retrieved, fertilization, and mean number of embryos transferred when compared to the negative selection and the positive selection groups (Table VIII). The mean number of cells of all embryos available for transfer was significantly ($P \leq 0.05$) higher in the negative selection group when compared to the non-selection group. The mean number of cells of embryos transferred was significantly ($P \leq 0.05$) higher in the positive selection group when compared to the non-selection and the negative selection groups. Within the limits of significance, we had no grounds to reject the null hypothesis that both implantation ($P \leq 0.0503$) and pregnancy ($P \leq 0.0722$) rates had an homogeneous distribution among groups. The distribution of singleton and multiple pregnancies, however, was significantly ($P \leq 0.05$) different among groups (the percentage of multiple pregnancies was higher in negative selection cycles, in which the slowest cleaving embryos were selected for transfer). No significant differences in fetal wastage (number of gestational sacs re-absorbed and fetuses miscarried) among groups were observed.

A further analysis of data was performed so as to know the outcome of pregnancy when combining both the type of embryo selection and maternal age in cycles reaching embryo transfer (Table IX). A significantly ($P \leq 0.05$) higher implantation rate was found in women <35 years of age when compared to women ≥35 years of age in non-selection cycles. A general tendency to lower implantation and pregnancy rates and higher percentages of fetal wastage was observed in women ≥35 years old.

Discussion

The present study shows that sex selection may be inadvertently performed in IVF–embryo transfer programmes when selecting for high quality embryos at the fresh transfer cycle. In particular, the present data show that, in cycles resulting in singleton births, the male to female ratio is significantly shifted toward the female (88.8%) and to the male (90.0%) when selecting for transfer the slowest or the fastest cleaving embryos respectively. However, contrary to appearances it seems that the mechanism by which this selection process operates is not entirely dependent on cleavage rate of embryos *in vitro*. In fact, (i) only a weak (Pergament *et al.*, 1994) or non-significant (this study) relationship between embryo cleavage rate and sex ratio of the resulting babies has been reported to date; and (ii) no shift on sex ratio was observed in cycles resulting in multiple births when either the slowest or the fastest cleaving embryos were transferred.

In contrast, the present data suggest that maternal age may interact with embryo cleavage rates and these together affect sex ratio at birth. In fact, sex ratio was significantly shifted toward the female (71.4%) and to the male (62.7%) in women ≥35 and <35 years of age respectively, despite the type of embryo selection performed for transfer being homogeneously distributed between groups (see Table VI). This age effect was also apparent when data were analysed combining both type of embryo selection and maternal age. In non-selection cycles

resulting in singleton births, sex ratio was skewed in favour of girls (75.0%) and in favour of boys (83.3%) in women ≥35 and <35 years of age respectively (see Table IX). In other words, maternal age appears to be an independent variable capable of affecting, in itself, sex ratio of IVF babies at birth. It is possible that oocytes from middle-aged and younger women are more likely to be fertilized by X- and Y-chromosome-bearing spermatozoa respectively. A possible mechanism explaining an age-associated differential fertilization may be based on a shift in either the production or function of X-chromosome and Y-chromosome-bearing spermatozoa as a man ages (maternal and paternal age are closely related factors). Alternatively, endometria from middle-aged women may select against males whereas some endometria from women <35 years of age may select against females, and others may be receptive to most embryos irrespective of their sex (these women would tend to have multiple pregnancies). That is to say, human endometria may be favourable, indifferent or hostile to either fast-cleaving or slow-cleaving embryos depending on maternal age. Although it is not possible from the present data to discriminate between these alternatives, the facts that (i) women ≥35 years of age exhibit a delay in endometrial development after ovarian stimulation with clomiphene citrate and/or exogenous gonadotrophins (Sterzik *et al.*, 1988); and (ii) the percentage of X- and Y-chromosome complements obtained after fusion with golden hamster oocytes is not significantly related to the man's age (Martin and Rademaker, 1992), support the second hypothesis. It is worth mentioning that in natural conceptions, maternal age is not an important cause of variation of human sex ratio at birth [see James (1987) for a review]. Sex ratio is affected by many biological, psychological, social -cultural, and geographic factors with complex associations between them. Such a high number of factors and interassociations may conceal any possible effect of maternal age on sex ratio. Many of these factors, such as timing of fertilization and/or length of the follicular phase (Weinberg *et al.*, 1995), are, however, under control in non-randomly selected IVF–embryo transfer patients.

It is of particular interest that embryos from the negative selection group did not show reduced implantation and pregnancy rates when compared to embryos from the non-selection and the positive selection groups. Conversely, they even showed a higher probability of multiple pregnancy when compared to the other two groups. This is in apparent contradiction to the well established axiom in human reproductive biology that retarded embryos have a reduced capability for implanting and giving rise to a pregnancy (Edwards *et al.*, 1984; Huisman *et al.*, 1994; Tarín *et al.*, 1994). Notwithstanding this, it is necessary to bear in mind that retarded embryos were chosen for transfer because they had better morphology than their sibling fast-cleaving embryos. Furthermore, transfers of retarded embryos in these patients were elective. These transfers differ from those cases in which patients generate a population of retarded embryos and have, therefore, an inevitable transfer of only slow-cleaving embryos. In this context, the study of Waterstone *et al.* (1991) is worth noting, in which elective transfer of two embryos resulted in a higher pregnancy rate than the inevitable transfer of two embryos, i.e. where only two were available.

The final point to be made concerns the possibility of performing 'natural' sex selection. This might be for reasons of personal preference or for preventing feticide, infanticide and homicide of females, or X-linked (male) disorders, or parental psychological distress, by controlling maternal age and the type of embryo selection. The present data show that the probability of correctly predicting female sex (0.79) is higher than that of male sex (0.48). Both values, however, are probably underestimates because of the retrospective character of the present study. In fact, most misclassifications came from cycles resulting in multiple pregnancies, cycles in which no embryo selection was performed or from cycles in which the fastest cleaving embryos were transferred into uteri of women ≥35 years of age. Prediction power may be gained if longer-term cultures (to the morula or the blastocyst stage) are used. At these stages, differences in embryo development and/or cleavage rates would be more apparent than on day 2. Embryo culture until day 4 appears to be a good alternative, since implantation and pregnancy rates are not reduced when compared to day 2 and day 3 cultures (Huisman *et al.*, 1994). Nature may also collaborate further to guarantee the correct prediction of female sex at birth. It is well known that in human beings a higher rate of loss of male as compared to female fetuses occurs during the first (Kellokumpu-Lehtinen and Pelliniemi, 1984), second (Carles *et al.*, 1991; Jakobovits, 1991; Chambers *et al.*, 1993) and third trimester of pregnancy (Carles *et al.*, 1991). 'Natural' sex selection, however, is limited by the fact that it predicts reduced implantation and pregnancy rates when the slowest cleaving embryos and the fastest cleaving embryos are transferred to women <35 and ≥35 years of age respectively. A prospective study is required to confirm the present results and to afford more realistic odds to couples wanting to use their right to choose the sex of their own offspring.

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