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ASSISTED REPRODUCTION

Blastocyst transfer and gender: IVF versus ICSI

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Abstract
Purpose To investigate the difference in sex ratio in our centre after day 5 embryo transfer among neonates delivered after fresh *in vitro* fertilization (IVF), fresh intra cytoplasmatic sperminjection (ICSI) and frozen embryo replacement (FER) compared to expected sex ratio in normal population.
Methods Retrospective data after IVF/ICSI/FER therapy from 1995 – 2007 and data from the literature on similar subjects.
Results Among a total of 420 births after day 5 transfer, there were 225 male and 195 female babies. However, after ICSI alone 30 were female and 21 male. Adding all available data from the literature on gender ratio after long term culture, the ratio of male births to total births (0.545) was significantly (p=0.004) different from the expected ratio (0.515)
Conclusions It appears that significantly more males are born than expected after day 5 transfer in IVF. However, a subgroup analysis in our data comparing IVF, ICSI and FER revealed more females born after ICSI and FER, suggesting that further research is needed in this area.

Keywords Blastocyst culture · IVF/ICSI · Sequential culture media · Sex ratio

Capsule Day 5 embryo transfer after *in vitro* culture leads to significantly more male babies than expected in normal population, with differences between IVF and ICSI.

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Introduction

Embryo selection for transfer in IVF/ICSI therapy is typically based on morphological criteria and rate of development. In animals, it has been demonstrated that the fastest growing embryos are more commonly male [22]. Several hypotheses have been addressed trying to explain this finding. Differences have been found regarding culture media conditions [12, 13], the maturation culture period of the oocyte [1], sperm damage [4] and epigenetic events which may modulate the difference between speed of development, metabolism and transcription, observed during preimplantation development between male and female embryos [5].

Some earlier studies in human ART (assisted reproduction technology) have shown that blastocyst transfer may favour male offspring [17–21, 24, 29] while others did not come to the same conclusion [3, 16, 26, 27, 30]. However, reporting criteria are unclear as to selection between IVF and ICSI. Either they report only on IVF patients or a mixed group of IVF and ICSI patients.

Materials and methods

This is a retrospective study from the IVF unit in Tromsø including all patients who gave birth after day 5 transfer

Table 1 Babies born after day 5 transfer, IVF unit Tromsø, 1995–2007

	Day5 ET	Babies born	Females	Males	Twins	Triplets	Quadrouplets
1995–2007	215	294	134	160	74	1	1
2005–2007	105	126	61	65	20[a]	1[a]	-
sum, all	**320**	**420**	**195**	**225**	**94**	**2**	**1**
ICSI							
1995–2007	**44**	**51**	**30**	**21**	**3**	**-**	**-**
FER, blc							
2005–2007	**11**	**12**	**9**	**3**	**-**	**-**	**-**

[a] There were three monozygotic twins in the period from 2005–2007

from 1.1.1995–31.12.2007. Transfers earlier than day 5 were not included, and only sequential culture media were used. During the study period the sequential culture media were refined and the stimulation protocols changed slightly over the years; long downregulation with GnRH agonist and ovarian hyperstimulation with uFSH (urinary follicle stimulating hormone) or recFSH (recombinant FSH) being the main stimulation protocol. ICSI was started in 1996, but in the beginning blastocyst culture was not performed on these patients. DET (double embryo transfer) was the routine until 2005. Freezing of surplus blastocysts and eSET (elective single embryo transfer) was initiated in October 2005.

Studies in the literature about gender and IVF/ICSI/FER were identified by searching in medical databases and public browsers with relevant keywords.

Statistics

Statistical analyses were performed using contingency tables for χ^2-test. $P \leq 0.05$ was taken as significant.

Results

The ratio of male births to total births in Northern Europe and America is expected to be 0.515, varying from 0.5087 to 0.5173 [11]. The sex ratio in our data was 0.55 after day 5 transfer in IVF in contrast to 0.41 in ICSI and 0.25 in FER, although the study groups were small (Table 1). Including all data on sex ratio and long term culture in IVF therapy from available literature (Table 2), the sex ratio was significantly different ($p=0.0044$) compared to the expected ratio. The p-value varied from $p=0.0006$ to $p=0.0084$ for an expected ratio of 0.5087 to 0.5173, respectively.

Discussion

This study covers a 12 years period focusing on gender outcome after day 5 transfer in IVF, ICSI and FER. Conflicting results in the literature about sex ratio after long term culture gave the stimulus to analyse the results from our clinic, which has several years of experience

Table 2 Babies born after day 5 (4–6) transfer, published to date

	Total babies born	Females	Males	Sex ratio
Menezo et al. 1999	383	158	225	0.587
Anderson et al. 2001	150	67	83	0.553
Kausche et al. 2001	163	71	92	0.564
Meintjes et al. 2001	402	168	234	0.582
Mercader et al. 2001	125	44	81	0.648
Rodriguez et al. 2001	82	35	47	0.573
Milki et al. 2003	163	66	97	0.595
Schwärzler et al. 2004	357	169	188	0.526
Richter et al. 2006	1,184	591	593	0.500
Luna et al. 2007	610	258	352	0.577
Csokmay et al. 2008	120	58	62	0.516
Weston et al. 2008	150	77	73	0.486
Hentemann et al. 2009	420	195	225	0.535
sum literature	**4,309**	**1,957**	**2,352**	**0.545**

(since 1994) with long term culture in human IVF, always using sequential culture media [6, 7, 14, 15].

We performed embryo transfer between day 2 and day 5. Only day 5 transfers were included in this study to insure, that at least small blastocysts or morulae were transferred and only the fastest growing embryos. Up to 2000, the embryos developed slower than in the refined media used thereafter. As a result, day 5 transfers during 1995 to 2000 included morulae. Due to the hypothesis that male embryos develop faster, these patients were included. Like in other studies we report an excess of males among infants born after IVF (Table 2). Adding all results from studies on this topic the difference is significant. Moreover, in our data we found more females born after ICSI and FER. However, our study groups are small and there is a need to expand these data in order to reach a firm conclusion.

In normally conceived pregnancies, the number of males exceeds that of females by a few percent at birth. In Europe and North America the male to female ratio (calculated as male live births divided by total live births) varies from 0.5087 in Mexico to 0.5173 in Greece [11]. Since all the studies published in English literature on sex ratio and long term embryo culture (Table 2) were from North America or Europe, we used the expected sex ratio of the study by Grech et al. to compare our data [11].

The results about sex ratio after ART are conflicting and interestingly the studies published so far do not differentiate between IVF and ICSI. They either find more males born after fresh blastocyst transfer compared to spontaneous conception [19] or compared to day 3 transfer [8, 17, 21], or they show no differences neither in sex ratio nor in growth rate of embryos [9, 23, 26–28, 30–32].

Alfarawati et al did a cytogenetic analysis of human blastocysts and found that the majority of developmentally advanced blastocysts were male (3:1 ratio of male to female) [2]. Dumoulin et al. found a clear sex related growth difference in a study on growth rate of human preimplantation surplus blastocysts [10]. The sex related difference was significantly greater in ICSI than in IVF embryos.

ICSI is the method of choice in cases with reduced semen quality. Sperm DNA damage is associated with significantly lower IVF pregnancy rate [33, 34]. In an early study of Ray et al. on fresh surplus embryos after *in vitro* fertilization [25], 63 % of the blastocysts examined, were female. However, the number of cells in male embryos was significantly greater, pyruvate, glucose uptake and lactate production was significantly higher in male embryos. From the differences in the number of cells they extrapolated that female embryos are approximately 4.5 h delayed in their development from day 2 onwards compared with male embryos. They suggest that differences between males and females occur early, either at fertilization or during the first or second cleavage. FER is done with the "next best" embryos, the best ones being transferred in a fresh cycle. Day 5 freezing and FER implies a combination of selection by morphology and development (after IVF or ICSI with the respective implications) plus survival after thawing.

Our findings suggest that the sex ratio can be influenced by selecting embryos on the basis of morphological characteristics and the techniques used. However, there is a need for more research in this area.

Conclusion

Studies in humans and animals provide the biological evidence for differences between the different techniques used in ART, which could lead to different outcome than expected in normal population. Our data calls for re-examination of this question as it may represent yet another influence that human ART has, based on the techniques employed.

Conflict of interest There is no conflict of interest for any of the submitting authors in reference to the submitted material.

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