

# Paternal contribution to embryonic competence

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**Introduction** The effect of paternal characteristics on embryo development and the outcome of preimplantation genetic testing for aneuploidy have not been extensively explored. This study investigates the association of sperm parameters, insemination, and extraction techniques, with the rate of embryo aneuploidy. This study sought to evaluate the association between male factor infertility and embryo aneuploidy.

**Material and methods** Patients underwent in vitro fertilization using intracytoplasmic sperm injection, with preimplantation genetic testing for aneuploidy. Patients were divided into four groups by sperm parameters: Group A: oligozoospermia (sperm concentration <10 million, morphology > 4%); Group B: teratozoospermia (sperm concentration >10 million, morphology <4%); Group C: oligozoospermia and teratozoospermia (sperm concentration <10 million, morphology <4%); Group D: controls. Additionally, couples were divided into three categories by days of abstinence: Group A: <2; Group B: 2-7; and Group C: >7.

**Results** A total of 4108 in vitro fertilization cycles with preimplantation genetic testing for aneuploidy were analyzed. After controlling for parental age and follicle count, the rate of embryo aneuploidy was not affected by duration of abstinence, sperm parameters, or the source of the sperm sample.

**Conclusions** Numerous factors related to sperm source and quality were evaluated, and a minimal influence on the rate of embryo aneuploidy was observed.

**Key Words:** aneuploidy ↔ male infertility ↔ preimplantation genetic testing ↔ sperm ↔ testicular sperm extraction

## INTRODUCTION

It is estimated that up to fifty percent of infertility cases arise solely or in part from sperm abnormalities [1]. Semen analyses provide a non-invasive and cost-effective method of evaluating male fertility, and are predictive of clinical outcomes [1]. Despite the growing use of preimplantation genetic testing for aneuploidy by in vitro fertilization clinics, the paternal contribution to the chromosomal competency of embryos remains unclear. This study investigates the association of sperm parameters, insemination, and extraction techniques, with the rate of embryo aneuploidy. Elucidating the paternal impact on the rate of embryo aneuploidy may enable reproductive specialists to better predict cycle

outcomes and consult with couples about their reproductive options.

The effect of maternal characteristics on the outcome of preimplantation genetic testing for aneuploidy has been extensively explored; however, male-based aneuploidy studies are limited and have primarily focused on modes of sperm extraction and/or fertilization techniques [2, 3, 4]. The influence of the genetic composition of sperm on embryo ploidy status remains highly contested among reproductive specialists [3–9]. Gianaroli et al. reported a high rate of monosomies and trisomies in embryos inseminated by intracytoplasmic sperm injection with sperm derived from testicular sperm extraction or testicular sperm aspiration [10]. Coates et al. demonstrated a significantly higher

rate of aneuploidy in embryos derived from sub-optimal sperm as compared to normal sperm [11]. Abnormal sperm production and/or function have been attributed to genetic, anatomical, endocrine and epigenetic factors [2, 12, 13, 14]. Increased aneuploidy has been demonstrated in sperm from men with oligozoospermia, asthenozoospermia, teratozoospermia, and azoospermia as compared to men with normal semen analyses [12, 15, 16, 17].

In this study we evaluate the paternal contribution to embryonic competence in couples undergoing in vitro fertilization, intracytoplasmic sperm injection and preimplantation genetic testing for aneuploidy. The rate of embryo aneuploidy, as a function of semen analysis parameters, duration of abstinence, and sperm extraction techniques, was investigated. The study's findings are anticipated to provide clinicians with information on the paternal contribution to embryo aneuploidy in patients pursuing in vitro fertilization treatment.

## MATERIAL AND METHODS

A single center, retrospective cohort analysis was conducted to evaluate infertility patients who underwent an in vitro fertilization cycle between November 2010 and March 2017, and who underwent trophectoderm biopsy of at least one embryo followed by preimplantation genetic testing for aneuploidy with quantitative polymerase chain reaction or next-generation sequencing. Only the first in vitro fertilization cycle with preimplantation genetic testing for aneuploidy per couple was included in the study. Donor sperm cycles were excluded from this study. The primary outcome of the study was the rate of embryo aneuploidy.

### Stimulation protocol

Patients underwent standard controlled ovarian hyperstimulation for in vitro fertilization with a down-regulation protocol with leuprolide acetate, an antagonist protocol, or a Microflare protocol [18]. Oocyte maturation was induced with recombinant human chorionic gonadotropin alone (Ovidrel 250 mg; EMD Serono) or with a 'dual trigger' using leuprolide acetate 2 mg (Lupron; AbbVie Laboratories) and human chorionic gonadotropin 1,000 IU (Novarel; Ferring Pharmaceuticals). Patients underwent vaginal oocyte retrieval under ultrasound guidance 36 hours after the trigger injection, and the oocytes were inseminated via Intracytoplasmic sperm injection as per protocol for patients undergoing preimplantation genetic testing for aneuploidy.

## Laboratory Procedures

### Embryo culture and biopsy techniques

On day 3 of embryo development, all embryos underwent laser-assisted hatching using a 200–300  $\mu$ s pulse ZILOS-tk Laser (Hamilton Thorne Biosciences) to create a 25–30  $\mu$ m opening in the zona pellucida to facilitate posterior trophectoderm herniation.

### Embryo culture and genomic screening

Blastocyst trophectoderm biopsies were performed on day 5 and/or day 6 of development, contingent upon morphologic eligibility (Gardner-Schoolcraft classification  $\geq 3BC$ ). Two to nine cells were sampled from the trophectoderm and analyzed using quantitative polymerase chain reaction or next-generation sequencing. Preimplantation genetic testing for aneuploidy results were finalized within 14 days of biopsy and were classified as 'euploid', 'aneuploid' (including mosaicism and/or segmental aneuploidy detected when next generation sequencing was performed), or uninterpretable.

### Cryopreservation and rewarming techniques

Following trophectoderm biopsy, the embryos were vitrified using a slightly modified Cryotop method [19]. For rewarming, the Cryotop was removed from the liquid nitrogen and placed in 1.0 mol/L sucrose in tissue culture medium 199 + 20% synthetic serum substitute at 37 °C. After 60 seconds, embryos were moved into 0.5 mol/L sucrose in tissue culture medium 199 + 20% synthetic serum substitute at room temperature for 3 minutes. Last, embryos were washed for 5 min and then for 1 min with tissue culture medium 199 + 20% synthetic serum substitute at room temperature [18]. After the embryos were rewarmed, the appearances of the blastomeres and zona pellucida, and the ability of the blastocoel to re-expand, were assessed to confirm embryo survival. Degenerated embryos were cataloged as non-surviving.

### Frozen embryo transfer cycle

Cycles with a least one blastocyst deemed to be euploid and available for frozen embryo transfer cycle were included in the study. The patient endometrium was synthetically-prepared with the administration of estradiol orally for 7 to 21 days (Estrace; Teva Pharmaceuticals), by transdermal patch (Estraderm) or intramuscular administration every other day

(Estradiol Valerate 4 mg IM). Once an endometrial thickness of  $\geq 7$  mm was documented sonographically, progesterone supplementation was initiated with intramuscular Progesterone in oil (Watson Pharmaceuticals) 50 to 100 mg per day, or by combined oral and vaginal routes (Prometrium, Crinone, or Endometrin). On day 5 of the progesterone supplementation, a single euploid embryo was transferred to the uterus under ultrasound-guidance.

### Variable definitions

**Paternal age:** Male patients were grouped by age according to criteria set by the Society for Assisted Reproductive Technology: <35, 35–37, 38–40, 41–42, and >42.

**Sperm parameters:** Patients were divided into four main groups based on semen parameters: Group A: oligozoospermia (sperm concentration <10 million, morphology >4%), Group B: teratozoospermia (sperm concentration >10 million, morphology <4%); Group C: oligozoospermia and teratozoospermia (sperm concentration <10 million, morphology <4%); Group D: non-male factor controls.

**Lengths of sexual abstinence:** Embryo aneuploidy rates were evaluated among males with varying

lengths of sexual abstinence. The couples were divided into three categories: Group A: <2 days abstinence; Group B: 2–7 days abstinence; Group C: >7 days abstinence.

**Sperm source:** To compare embryo aneuploidy rates as a function of sperm source, semen samples were divided into two groups: Group A: sperm acquired from ejaculation; Group B: sperm acquired from testicular sperm extraction.

### Statistical analyses

Statistical analyses were performed using Statistic Applied Software version 9.4 (Statistic Applied Software Institute Inc., Cary, NC, USA). Univariate diagnostic tests were run using non-parametric Kruskal-Wallis and Mann Whitney tests. In order to ascertain the male contribution to the odds of embryo aneuploidy, a logistic regression was used to calculate binomial confidence intervals for the proportion of abnormal embryos over the total number of embryos biopsied. In order to eliminate inter-patient association bias resulting from multiple embryo transfers, only the transfer results from the first cycle were used in this analysis.

**Table 1.** Maternal and paternal demographic factors as well as semen characteristics

Variable	Category	N = 4108	Aneuploidy Rate %	P-Value*
Sperm Source	Testicular Sperm Extraction	67	46.4	.393
	Ejaculate**	3902	49.7	
Sperm Parameters	Oligozoospermia	44	43.8	.410
	Teratozoospermia	1243	51.0	
	Oligozoospermia & Teratozoospermia	136	49.5	
	Normospermia**	2685	49.3	
Days of Abstinence	<2 Days	1567	49.1	.742
	2-7 Days	2291	49.9	
	>7 Days**	250	49.4	
Oocyte Age	< 35	1452	35.3	<.001
	35 – 37	1045	43.9	
	38 – 40	978	57.9	
	41 – 42	426	77.2	
	> 42**	196	87.6	
Paternal Age	< 35	1346	41.0	<.001
	35 – 37	817	45.3	
	38 – 40	768	49.5	
	41 – 42	359	55.7	
	> 42**	818	62.8	
Maternal Follicle Count	Mean	12.19	49.8	<.001
	Median	11	50.0	
	Minimum	0	0	
	Maximum	55	100	
	Std Dev	6.89	±34.7	

\*P-Values are calculated by using non-parametric Kruskal-Wallis and Mann Whitney Tests

\*\*Reference Group

Note: Categorical sums exclude missing values

**Table 2.** Characteristics of sperm parameters, days of abstinence, and maternal demographics

Variable	Category	Aneuploidy Rate %	Odds Ratio 95% CI
Sperm Source	Testicular Sperm Extraction	46.4	.635 (.386–1.046)
	Ejaculate**	49.7	–
Sperm Parameters	Oligozoospermia	43.8	1.252 (.69–2.273)
	Teratozoospermia	51.0	.917 (.804–1.045)
	Oligozoospermia & Teratozoospermia	49.5	1.02 (.733–1.428)
	Normospermia**	49.3	–
Days of Abstinence	<2 days	49.1	.978 (.739–1.293)
	2–7 days	49.9	.881 (.673–1.153)
	>7 days**	49.4	–
Oocyte Age	< 35	35.3	14.54 (10.0–21.1*)
	35–37	43.9	13.04 (9.10–18.8*)
	38–40	57.9	6.763 (4.735–9.659*)
	41–42	77.2	2.25 (1.531–3.306*)
	> 42**	87.6	–
Paternal Age	< 35	41.0	1.07 (.873–1.311)
	35–37	45.3	1.036 (.846–1.269)
	38–40	49.5	1.069 (.879–1.3)
	41–42	55.7	1.02 (.799–1.3012)
	> 42**	62.8	–
Maternal Follicle Count	–	49.8	1.065 (1.055–.075*)

\*P-Values indicate  $P < .005$ 

\*\*Reference Group

## Regulatory approval

This retrospective study was approved by the Western Institutional Review Board. Patient information was anonymized and de-identified prior to analyses.

## RESULTS

A total of 4108 first in vitro fertilization cycles with preimplantation genetic testing for aneuploidy were analyzed between November 2010 and June 2018. Maternal and paternal demographic factors as well as semen characteristics for the cycles are listed in Table 1. Unadjusted rates of aneuploidy were significantly associated with maternal age ( $p < 0.001$ ), paternal age ( $p < 0.001$ ), and follicle count ( $p < 0.001$ ). A multivariate logistic regression was performed to identify attributes most significantly associated with embryonic aneuploidy. After controlling for parental age and follicle count, embryonic ploidy status did not appear to be affected by the number of days of abstinence, sperm parameters, or the source of the semen sample (Table 2).

A higher maternal follicle count was significantly associated with a lower chance of embryo aneuploidy (OR = 1.065, CI = [1.055–0.075]). There was no difference in aneuploidy rates when sperm source (testicular sperm extraction or ejaculate) used for insemination was evaluated (OR = .635, CI = [0.386–1.046]).

## DISCUSSION

In a growing era of intracytoplasmic sperm injection and preimplantation genetic testing for aneuploidy, this study demonstrated that the risk of embryo aneuploidy has minimal associations with sperm parameters, number of days of abstinence, or testicular sperm extraction technique. Moreover, these data suggest that chromosomally normal embryos can be obtained in cases of severe male factors through the use of advanced technologies, even those involving surgical sperm extraction.

The increased rate of embryonic aneuploidy with advanced maternal age has been well-established [20, 21, 22]. However, as paternal age rises with each generation [23], our data suggest that abnormal sperm parameters, namely oligozoospermia and/or teratozoospermia, do not appear to be associated with increased embryo aneuploidy. The findings of this study do not corroborate with those of Coates et al. who reported an increased rate of sex chromosomes abnormalities in embryos derived from men with oligozoospermia as compared to men with normal sperm [13]. Our findings are in accordance with researchers who reported poorer fertilization rates and embryo development but no significant reduction in the rate of euploidy in embryos derived from men with severe male factors [24].

Medical literature is replete with articles addressing the effects of ejaculatory abstinence on semen

analysis parameters. Improved sperm motility was found in semen produced after two hours of abstinence as compared to abstinence lasting several days [25]. Semen volume and sperm concentration has been found to improve with increased duration of ejaculatory abstinence [26]. In a systematic review of 28 recent publications, longer abstinence was associated with increases in semen volume and sperm count but the effects of abstinence on sperm motility and morphology were inconclusive [27]. Our study suggests the duration of ejaculatory abstinence to be clinically inconsequential as we demonstrated no difference in the rate of embryo aneuploidy among the cohorts segregated by days of abstinence.

Many of our findings are consistent with the current literature. Sperm extraction techniques including testicular sperm extraction have been shown to be effective and result in viable pregnancies, suggesting sperm extraction and insemination with intracytoplasmic sperm injection does not appear to adversely affect the rate of embryo euploidy [28]. These varying conclusions suggest that the male contribution to embryonic competence, if any, is nuanced and

multifactorial. Our study has the advantage of its large size (data from over 4000 in vitro fertilization cycles were analyzed) and the fact that all cases were conducted at a single institution. In addition, only first in vitro fertilization cycles were evaluated. The study does have important limitations. Its retrospective design limited our ability to exclude potential confounding variables such as duration of infertility, body composition of each partner, and female diagnoses. Furthermore, our center replaced quantitative polymerase chain reaction or next-generation sequencing during the time frame of the study which might have influenced the proportion of embryos deemed aneuploid.

This study adds to current literature suggesting nominal, if any, influence of particular sperm parameters on rates of embryo aneuploidy. Our findings confirm that even in cases of severe male factors and testicular sperm extraction, favorable embryo euploid rates can be achieved.

#### CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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