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Original Article

Sex Ratio of Live Births Following Fresh Versus Cryopreserved-Thawed Embryo Transfer in Invitro Fertilization: A Retrospective Study

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Abstract:

Background: Human sex is genetically determined at fertilization; however, emerging evidence suggests that assisted reproductive technologies (ART) may influence the secondary sex ratio (SR). Factors related to parental characteristics, laboratory practices, and embryo handling have been implicated, though findings remain inconsistent. This study aimed to compare the secondary sex ratio of live births following fresh versus frozen–thawed single blastocyst transfer.

Methods: We conducted a multicenter retrospective cohort study reviewing IVF records from Kingswill Specialist Hospital, Lagos, and referrals to the 68 Nigerian Army Reference Hospital and Lagos University Teaching Hospital. Records from 1 January 2017 to 31 December 2025 were assessed. Women who achieved live birth following single blastocyst transfer in either fresh or frozen–thawed IVF cycles were included.

Results: Of 159 records screened, 144 met eligibility criteria and were analyzed (72 fresh and 72 frozen–thawed cycles). Fresh embryo transfer was associated with a higher proportion of male live births compared with frozen–thawed transfer (69.4% vs 45.8%), whereas frozen–thawed cycles yielded a higher proportion of female infants (54.2%).

Conclusion: Fresh single blastocyst embryo transfer was associated with a higher male-to-female live-birth ratio compared with frozen–thawed blastocyst transfer, despite transfer at an equivalent developmental stage. These findings suggest that embryo cryopreservation may be associated with differences in sex ratio following IVF.

Keywords: Sex Ratio; Fresh IVF; Frozen-Thawed Embryo; Maternal Age; Body Mass Index

Introduction

There is usually not so much disparity in sex ratio (SR) in a population. This is an observation that has intrigued many evolutionary scientists to believe that it is a form of natural selection to maintain a global gender equilibrium [1,2,3]. Ordinarily without intervention, it has been reported that in most populations that the sex ratio ranges from 102 males to every 100 female live birth [1,4,5]. Previous studies have suggested that this remarkable consistency notwithstanding, that SR can be affected by many factors including selective abortion or use of infanticide in some countries, maternal age at conception, race/ethnicity of father, frequency of sexual intercourse, birth order, family size, wars, stress, occupation of the parents, maternal hormonal treatments, harsh economic conditions, malnutrition, male infertility and environmental pollution [6,7]. Whether these factors or their own or in combination can skew the SR in favor of a particular gender or just a mere confounding variables is still a subject that is still yet to be thoroughly evaluated in many studies [1,4,7].

Conventionally, it is known that human sex is determined at birth by the sex chromosomes contributed by either parent (especially the Y-chromosome from the male partner) at fertilisation. Recently, there has been an increasing amount of evidence to suggest that procedures done during assisted conception may have a significant impact on sex ratio (SR).1 These authors have argued that several factors in whole or part may play a role in determining the sex ratio [1,4,6]. These include factors such as parental variables (paternal race and height, maternal age and body mass index, maternal ovarian reserve), method of fertilisation (invitro fertilisation versus intracytoplasmic sperm injection), stage of embryo at transfer (cleavage versus blastocyst),number of embryo transferred, type of IVF cycle (fresh versus cryopreserved gametes or embryos), medications used for controlled ovarian stimulation, male factor infertility, culture media and application of artificial intelligence during embryo monitoring and selection [1, 2, 3, 6].

The recent tremendous advances in assisted conception and development of facilities for vitrification and cryopreservation have contributed immensely to the treatment of infertile couples, by providing opportunities for gamete or embryo preservation for future use which invariably have relieved the anguish of the affected families [8]. Embryo cryopreservation has continued to be a reliable adjunct in many IVF laboratories today. There have been different findings in the literature on the effect of thawed embryo on sex ratio. While some argue that thawed blastocyst embryo transfer favors male live birth, others hold a contrasting opinion [10, 11]. Nagata and colleagues in their study following invitro fertilization and embryo transfer (IVF-ET) concluded that although they found no significant change in SR between fresh or frozen cycle, however they affirm that the rate of embryo development and its metabolism as well as selection process before cryopreservation may have an impact on the overall SR of live births [12].

Additionally, among the authors who found that SR is skewed in Favour of females following thawed blastocyst transfer, it has been suggested that this may be the effect of culture duration on the embryo. They opined that spermatozoa bearing Y-chromosome may be at increased risk of accelerated damage by temperature fluctuations and prolonged culture duration, as well as its increased expression of apoptotic genes in culture media. This synergistically may cause more demise of male embryos in utero in favour of the females [10, 13].

Contrary to the above, other authors support the hypothesis that male embryos grow faster and has higher metabolic rate than their female counterparts and are therefore selected more for vitrification and later transfer in frozen-thawed IVF cycle [1, 11]. They also observed that male embryos have better chance of survival following vitrification, reason of which is not yet understood [1, 11]. Therefore, there is increased sex ratio when frozen-thawed blastocyst is transferred compared to frozen-thawed cleavage stage embryo in invitro fertilization [11,14].

On the other hand, some of these researchers did not categorically state if the effect on SR was solely because of blastocyst transfer as most embryos were vitrified at blastocyst stage or is it the effect of vitrification process on embryo survival [14,15].

It is because of this conflicting evidence that this study aims to investigate the potential impact of on the secondary sex ratio of live births following fresh versus frozen–thawed single blastocyst transfer in Nigerian women.

Study Design and Setting

This was a nine-year retrospective study of patient records from Kingswill Specialist Hospital, Lagos, as well as patients referred to the 68 Nigerian Army Reference Hospital, Lagos, and Lagos University Teaching Hospital following in vitro fertilization (IVF) procedures. Medical records spanning 1 January 2017 to 31 December 2025 were reviewed.

Study Participants

The study population comprised women who underwent either fresh in vitro fertilization (IVF) cycles or frozen–thawed embryo transfer and achieved a successful clinical outcome at the participating centers, or who were referred for specialized obstetric care following successful embryo transfer. Inclusion was restricted to women who subsequently delivered a live-born infant at or beyond the threshold of fetal viability (≥28 weeks' gestation).

Inclusion and Exclusion Criteria

Criteria for Study Inclusion

Inclusion was restricted to women who subsequently delivered a live-born infant at or beyond the threshold of fetal viability (≥28 weeks' gestation).

Exclusion Criteria

Women were excluded if they experienced early pregnancy loss, had incomplete medical records, carried twins or higher-order multiple pregnancies, underwent IVF with elective sex selection, or delivered outside the participating study centers.

Clinical Outcome

The primary study endpoint was the sex ratio of live-born infants resulting from age- and parity-matched fresh versus frozen–thawed embryo transfer cycles.

Sample size calculation

The sample size was determined a priori using established statistical methods for binary outcomes.16 Calculations were based on achieving adequate statistical power to detect a difference in outcome proportions between the fresh and frozen–thawed embryo transfer groups.16 This approach indicated that a minimum of 49 participants per group was required for the analysis.

Ethics Approval

Ethical approval was obtained from the 68 Nigerian Army Reference Hospital Yaba, (NARHY) Ethics Review Board. All patient data were fully anonymized to ensure confidentiality throughout the study.

Data Analysis

Data were collected using study-specific proforma designed to capture relevant participant characteristics and clinical variables. The dataset was subsequently entered and analyzed using the Statistical Package for the Social Sciences (SPSS), version 29.0 (IBM Corp., Armonk, NY, USA). The sex ratio was expressed as the proportion of male live births per 100 female live births and presented as percentages. Associations between infant sex and categorical variables (maternal age, type of embryo transfer, and maternal booking body mass index) were assessed using the chi-square test or Fisher's exact test where appropriate.

The observed proportion of male births in the fresh-embryo and frozen-embryo transfer groups was compared with the expected population proportion of male births using +the exact binomial test. A two-sided p-value < 0.05 was considered statistically significant.

Univariate logistic regression analyses were performed to evaluate the association between sex ratio and each explanatory variable. Multivariable logistic regression models were subsequently constructed to adjust for potential confounding factors.

Results

A total of 159 patient records from the participating centers were screened for eligibility. Following application of the inclusion and exclusion criteria, 144 records were deemed eligible and included in the final analysis, with 72 participants allocated to each study group.

The distribution of neonatal sex differed by embryo transfer type, with a higher proportion of male infants observed among pregnancies conceived through fresh IVF cycles (n =50 (69.4)

Vs 22(30.6%) females compared with frozen–thawed embryo transfers with 39(54.2) females and 33(45.8%) males-Table 1. This implies that the sex ratio in the fresh-embryo group was skewed towards males, but in the frozen-embryo group it was roughly as expected in the general population (54.2%). In other words, fresh embryo transfers appear to result in a male-skewed sex ratio, unlike frozen-embryo transfers.

The mean maternal age was comparable between the frozen–thawed embryo transfer and fresh embryo transfer groups (34.27 ± 3.2 years vs 34.32 ± 2.9 years, respectively). Similarly, mean maternal body mass index did not differ materially between the two groups (28.22 ± 2.4 kg/m² vs 28.35 ± 3.6 kg/m²). Median parity was 0 (interquartile range [IQR]: 0–1) in the frozen–thawed cohort and 0 (IQR: 0–2) in the fresh embryo cohort. No statistically significant differences were observed between the groups for these baseline characteristics. Table 2.

Table 1. Baseline characteristics and distribution of sex ratio according to maternal age, IVF type, paternal age, maternal body mass index, and duration of infertility.

Variables	Males (n=89)	Females (n=55)	X^2	p-value
Maternal Age (years)				
≤30				
31-35	45(63.4)	26(36.6)	2.421*	0.341
36-40	27(58.7)	19(41.3)		
>40	12 (63.2)	7(36.8)		
	4(57.1)	3(42.9)		
Paternal age			2.443*	0.225
≤30	3(75.0)	1(25.0)		
31-35	22(56.4)	17(43.6)		
36-40	41(58.6)	29(41.4)		
>40	23(74.2)	8(25.8)		
Embryo type			4.252#	**0.002**
Fresh embryo	50(69.4)	22(30.6)		
Frozen-thawed embryo	39(54.2)	33(45.8)		
Maternal booking BMI			1.286#	0.424
Normal weight	33(60.0)	22(40.0)		
Overweight	30(55.6)	24(44.4)		
Obesity	26(74.3)	9(25.7)		
Duration of infertility (years)				
1-5	36(63.2)	21(36.8)		
6-10	28(60.9)	18(39.1)		
>10	25(60.9)	16(39.1)		

*Fisher's exact test

#Chi square

Male and *Female* represent the counts of male and female live-born infants, respectively.

Table 2. Summary of maternal demographic and clinical characteristics: mean age, BMI and Median parity.

Parameters	Frozen Embryo cohorts	Fresh Embryo cohorts	p-value
Mean maternal Age ±SD	34.27±3.2	34.32±2.9	0.351
Parity (median, IQR)	0(0-1)	0 (0-2)	0.389
Mean maternal BMI	28.22±2.4	28.35±3.6	0.286

Table 3. Univariate and Multivariable Logistic Regression Analysis of Factors Associated with Offspring Sex (Male Birth)

	Univariate		Multivariate	
	Odd ratio (95% CI)	p-value	Odd ratio (95% CI)	p-value
Maternal age				
≤30	1			
31-35	0.552(0.237-1.130)	0.231		
36-40	0.662(0.430-1.237)	0.302		
>40	0.745(0.412-1.457)	0.540		

Table 3. *Cont*

Embryo type				
Fresh embryo	1	**0.002**	1	**0.002**
Frozen-thawed embryo	3.670(2.445-6.357)		3.5661(2.315-6.456)	
Paternal age				
≤30	1			
31-35	0.332(0.323-1.213)	0.442		
36-40	0.362(0.620-1.317)	0.431		
>40	0.441(0.455-1.472)	0.428		
Maternal Body mass index				
Normal weight				
Overweight	1			
Obesity	0.216(0.853-1.676)	0.332		
	0.574(0.434-1.425)	0.321		

Discussion

Recent literature has reported inconsistent findings regarding the influence of assisted reproductive technologies on population sex ratios. In the present study, a higher proportion of male live births was observed following fresh IVF cycles compared with frozen–thawed embryo transfer cycles. This association persisted after adjustment for key parental and clinical characteristics, including paternal age, maternal age, and maternal body mass index. The robustness of this finding after multivariable adjustment suggests that embryo cryopreservation may be independently associated with variations in sex ratio. However, given the observational design, these findings should be interpreted cautiously, and further prospective studies are warranted to elucidate the underlying biological mechanisms and to confirm the reproducibility of this association. The findings of the present study differ from those reported by **Du et al**., who observed a higher proportion of male live births following frozen–thawed IVF cycles. This discrepancy may be attributable to important methodological differences between the studies.[17] Specifically, **Du et al**. compared frozen–thawed single blastocyst transfers with frozen–thawed single cleavage-stage embryo transfers, whereas the current study restricted analysis to single blastocyst transfers in both fresh and frozen–thawed cycles.[17] By standardizing embryo developmental stage across exposure groups, the present analysis reduces potential confounding related to embryo stage at transfer, thereby providing a more comparable assessment of the association between embryo cryopreservation and sex ratio. These methodological differences should be considered when interpreting and comparing findings across studies.

Also, in contrast to our findings**, Nagata et al**. reported no significant differences in sex ratio according to fertilization method, timing of embryo transfer, or whether embryos were transferred fresh or after cryopreservation.[12] Nonetheless, the authors postulated that the increasing use of intracytoplasmic sperm injection (ICSI) and blastocyst-stage transfers may have the potential to influence population sex ratios over time. These observations suggest that while individual cycle characteristics may not consistently demonstrate measurable effects, evolving assisted reproductive technologies and practice patterns could exert subtle, cumulative influences on sex ratio outcomes. Differences in study populations, laboratory protocols, and analytical approaches may further contribute to the variability observed across studies.

Additionally, **Perlman *et al.* (2021)** reported a higher proportion of male live births following single blastocyst transfer compared with cleavage-stage transfer.[10] Although their analysis did not directly

compare fresh and frozen IVF cycles, the authors proposed that sex-specific differences in embryonic development may partially explain this observation. Specifically, male embryos may exhibit more rapid developmental kinetics and higher metabolic activity than female embryos, increasing their likelihood of selection for blastocyst transfer and vitrification. They further observed that male embryos appeared to have higher post-vitrification survival rates, although the underlying biological mechanisms remain unclear. These hypotheses underscore the potential role of embryo developmental dynamics and laboratory selection processes in influencing sex ratio outcomes following assisted reproductive technologies.

Conclusion

In conclusion, this study demonstrates that fresh single blastocyst embryo transfer is associated with a higher proportion of male live births compared with frozen-thawed single blastocyst transfer, despite both interventions involving transfer at the same developmental stage. This finding suggests that factors intrinsic to the fresh versus cryopreserved embryo environment, rather than blastocyst stage alone, may influence offspring sex ratio following IVF. The persistence of this association after adjustment for relevant parental and clinical characteristics supports a potential independent effect of embryo cryopreservation on sex ratio outcomes. Further large-scale, prospective studies incorporating embryological, molecular, and laboratory-level variables are warranted to elucidate the biological mechanisms underlying these observations and to clarify their implications for assisted reproductive practice.

This study has several strengths. It was conducted across multiple centers and involved a relatively homogeneous population of Black women, thereby reducing population heterogeneity and enhancing internal validity. Important clinical and laboratory factors including infertility etiology, ovarian stimulation protocol (agonist versus antagonist), fertilization method, (insemination versus ICSI), embryo culture media, and incubator type were not evaluated and may have influenced embryo development and selection. Additionally, the retrospective design limits causal inference and is susceptible to residual confounding. These limitations should be considered when interpreting the findings and highlighting areas for future prospective investigation.

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