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ASSISTED REPRODUCTION TECHNOLOGIES

The use of fresh compared to frozen ejaculated sperm has no impact on fresh embryo transfer cycle reproductive outcomes

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Abstract

Purpose To compare the reproductive outcomes of fresh embryo transfer (ET) cycles utilizing fresh versus frozen ejaculated sperm.

Methods First autologous fresh embryo transfer cycles at a single high-volume academic institution between 2013 and 2019 were retrospectively reviewed. IVF cycles using ejaculated sperm were included, and cycles using donor or surgically retrieved sperm were excluded. Sperm concentration was stratified as ≥ 5 and < 5 million/ml. The primary outcome was live birth, and the secondary outcomes were clinical intrauterine pregnancy (IUP) and miscarriage. A multivariable logistic regression model for the aforementioned outcomes was adjusted *a* priori for sperm concentration as well as maternal and paternal age.

Results A total of 6128 couples were included. Of these, 5780 (94.3%) utilized fresh sperm, and 348 (5.7%) frozen sperm. A total of 5716 (93.2%) had sperm concentrations ≥ 5 million/ml and 412 (6.7%) had sperm concentrations < 5 million/ml. On multivariable logistic regression, the use of freshly ejaculated sperm was not associated with significantly different odds of clinical IUP, miscarriage, or live birth when compared to cycles using frozen sperm.

Conclusion For couples conceiving via fresh ET, the use of fresh versus frozen ejaculated sperm is not associated with reproductive outcomes.

Keywords Ejaculated sperm · Fresh sperm · Frozen sperm · In vitro fertilization

Introduction

The ability to fertilize oocytes via in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) has revolutionized the treatment of infertility for a variety of both male and female factors [1]. Ovarian stimulation treatments may be complex, with protocol adjustments made daily based on the patients' response. Due to the nature of this treatment, patients often receive short notice for when their oocyte retrieval will be scheduled, which may cause logistical challenges for both the patient and their partner [2, 3]. Male partners are typically asked to provide a fresh semen specimen on the day of oocyte retrieval to be used for oocyte fertilization. However, some male partners may have logistical or psychological concerns with providing a fresh semen specimen on short notice and therefore may instead choose to provide a semen specimen in advance as a frozen specimen.

Limited data exists assessing the use of fresh versus frozen ejaculated sperm in assisted reproductive technology (ART). It has been hypothesized that subsequent freezing and thawing of sperm may cause harm and negatively impact sperm viability and motility [4]. Given these concerns, early studies demonstrated a favorable benefit to fresh sperm in donors in some instances, such as in women undergoing intrauterine insemination (IUI), but no difference in others using IVF [5, 6]. Since then, numerous data have been published evaluating the use of fresh versus frozen sperm in men with oligozoospermia, cryptozoospermia, and nonobstructive azoospermia (NOA), with multiple recent systematic

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reviews demonstrating no difference in fresh or frozen sperm in men with NOA [7, 8]. However, since the advent of ICSI, there is a paucity of data examining the impacts of fresh versus frozen ejaculated sperm in men without known infertility or subfertility. A single study with a small sample size suggests no differences in implantation or rate of pregnancy; however, pregnancy loss and live birth outcomes were not clearly reported [9].

Given the absence of data examining fresh versus frozen ejaculated sperm in men without infertility, we sought to compare the reproductive outcomes of fresh versus frozen ejaculated sperm used during fresh embryo transfer (ET) cycles at a large high volume academic institution.

Methods

Study population

We reviewed all patients between January 1, 2013, and December 31, 2019, at the Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine undergoing controlled ovarian hyperstimulation. Only first, fresh autologous cycles with ejaculated sperm were included. Couples were excluded if oocytes were cryopreserved prior to fertilization, if donor sperm were used, if sperm concentration was < 100,000 sperm per milliliter as this subset of men has both been previously well studied and may represent a different protoplasm given their increased burden of disease or if the cycle outcomes were unknown [7, 8, 10]. Institutional Review Board approval was obtained from Weill Cornell Medicine (protocol number 19-06020283).

Demographic data and clinical data

All patient data were recorded into an electronic database. Age, race (White, Asian, Black, and other/unknown/declined) and maternal body mass index were included. Infertility diagnosis for the female was recorded using Society for Assisted Reproductive Technology (SART) categorizations, including idiopathic, anovulatory/polycystic ovarian, diminished ovarian reserve, tubal factor, uterine factor, endometriosis, and preimplantation genetic testing for monogenic disorders (PGT-M). Ejaculated sperm was recorded as frozen or fresh, and concentration (post-thaw if frozen) was recorded as > 5 million/ml or < 5 million/ml.

Clinical protocols

All included patients underwent ovarian stimulation with a flexible gonadotropin-releasing hormone (GnRH) agonist or GnRH antagonist based protocol with the starting dose determined based on weight, age, and ovarian reserve [11, 12]. Patients with diminished ovarian reserve received 0.1 mg estradiol patches (Climara) (Bayer HealthCare, Leverkusen, Germany) for ovarian priming in the preceding luteal phase for follicular synchronization. Ovulation was triggered with either an hCG (Novarel) (Ferring Pharmaceuticals Inc., Parsippany, NJ, USA) or Pregnyl (Merck, Whitehouse Station, NJ, USA) trigger or a dual trigger with hCG and a 4 mg GnRH agonist (leuprolide acetate) (Sandoz Inc., Princeton, NJ, USA) with hCG dosing based on a sliding-scale regimen once two leading follicles measure greater than 17 mm [13, 14]. Oocytes were retrieved 35–37 h after the ovulatory trigger. Embryos were evaluated on the morning of day 3 and day 5 and were graded as previously described [15].

Decisions regarding day to transfer embryos as well as the number of embryos to transfer were made by the treating physician in consultation with the patient and embryology laboratory. All patients received daily intramuscular progesterone 50 mg beginning the day after oocyte retrieval until 8–10 weeks of gestation. Patients who received a dual trigger with less than 3300 units of hCG were also prescribed a 0.1 mg estradiol patch (Climara) (Bayer HealthCare, Leverkusen, Germany) every other day until approximately 8 weeks of gestation. Serum hCG levels were assessed 10 days after embryo transfer, and transvaginal ultrasound was performed to confirm intrauterine pregnancy by 5–7 weeks of gestation.

Exposure definition

Our main exposure of interest was the use of frozen or fresh ejaculated sperm. We also stratified our cohort using sperm concentration as ≥ 5 and < 5 million/ml (severe oligozoospermia).

Outcome

Our primary outcome of interest was odds of live birth defined as the delivery of a live child born at ≥ 24 weeks of gestation. Secondary outcomes included odds of clinical intrauterine pregnancy (IUP) (defined as the observation of at least one intrauterine gestational sac on ultrasound) and miscarriage (defined a failed pregnancy after the observation of at least one intrauterine gestational sac on ultrasound).

Statistical analysis

Demographic data were reported with appropriate summary statistics (means with standard deviations (SD) and medians with interquartile ranges (IQR)). Data distribution was observed graphically using histograms. Between-group comparisons were completed as chi-square for dichotomous variables and *t*-test and/or ANOVA for continuous variables. Univariable and multivariable logistic regression were

completed for the outcome of interest with frozen sperm as the referent using *a priori* selected variables for multivariable analysis, including male age (continuous), female age (continuous), and sperm concentration (continuous). All analysis was completed with Stata v17 (StataCorp LLC, College Station, TX).

Results

Study cohort

Between 2013 and 2019, a total of 12,899 fresh autologous IVF cycles were performed. Of these, 2586 were excluded as their cycles were for upfront oocyte cryopreservation, 53 had a fresh transfer on a day other than day 3 or 5, 110 were natural cycle IVF, 856 had surgical sperm retrieval, 2372 had sperm counts < 100,000, and 794 used donor sperm or sperm source information was available in the EMR. Therefore, a total of 6128 couples were included. The median maternal age of the study cohort was 37 years (IQR 34–41), and the median paternal age was 39 years (IQR 35–43). The majority of couples ($n = 5780$, 94.3%) used fresh ejaculated sperm, and the remaining couples ($n = 348$, 5.7%) used frozen sperm.

Patient and cycle characteristics

Patient demographics are displayed in Table 1. The mean female age was comparable between groups ($p = 0.608$), but the mean male age was greater in the frozen compared to the fresh sperm group (41.0 years versus 39.2 years, $p < 0.001$). Differences were observed for both maternal race ($p < 0.001$) and paternal race ($p = 0.044$). For both maternal and paternal races, Asians and Blacks were more likely to use fresh sperm, but those with a paternal race recorded as White were more likely to use frozen sperm, and those with a maternal race as White were more likely to use fresh sperm. Overall sperm concentrations were greater in the fresh sperm group compared to the frozen group ($p < 0.001$), as was the total motile sperm count ($p < 0.001$).

The mean number of mature oocytes was similar for both groups ($p = 0.577$) (Table 2). All of the frozen sperm used ICSI ($p < 0.001$). The fertilization rate was comparable for fresh versus frozen sperm ($p = 0.307$). No differences were observed in embryo quality ($p = 0.251$ for

Table 1 Demographics and baseline factors ($n = 6324$)

	Fresh sperm ($n = 5780$)	Frozen sperm ($n = 348$)	*P*-value
Maternal age (mean, SD)	37.07 (4.50)	36.95 (5.01)	0.608
Paternal age (mean, SD)	39.22 (6.28)	41.03 (8.84)	< 0.001
Maternal BMI (mean, SD)	23.46 (6.45)	22.74 (5.98)	0.043
Maternal race (*n*, %)			< 0.001
Other/unknown/declined	1451 (25.1%)	124 (35.6%)	
White	3014 (52.1%)	165 (47.4%)	
Asian	1111 (19.2%)	49 (14.1%)	
Black	204 (3.5%)	10 (2.9%)	
Paternal race (*n*, %)			0.044
Other/unknown/declined	2394 (41.4%)	148 (42.5%)	
White	2560 (44.3%)	168 (48.3%)	
Asian	657 (11.4%)	23 (6.6%)	
Black	169 (2.9%)	9 (2.6%)	
Infertility diagnosis (*n*, %)*			
Idiopathic	475 (8.2%)	22 (6.3%)	0.208
Anovulatory	439 (7.6%)	20 (5.7%)	0.203
Dim. ovarian reserve	3478 (6.0%)	198 (56.9%)	0.226
Tubal factor	1062 (18.4%)	44 (12.6%)	0.007
Uterine factor	442 (7.6%)	16 (4.6%)	0.036
Endometriosis	612 (10.6%)	24 (6.9%)	0.028
Sperm concentration (*n*, %)			< 0.001
≥ 5 million/ml	5457 (94.4%)	259 (74.4%)	
< 5 million/ml	323 (5.6%)	89 (25.6%)	
Total motile count (million, mean, SD)	68.7 (72.6)	4.6 (13.0)	< 0.001

*May have one or more diagnosis

Table 2 Cycle characteristics based on fresh or frozen ejaculated sperm

	Fresh sperm ($n=5780$)	Frozen sperm ($n=348$)	*P*-value
Stimulation protocol (*n*, %)			0.829
GnRH antagonist	5127 (88.7)	310 (89.1)	
GnRH agonist	653 (11.2)	38 (10.9)	
No. mature oocytes (mean, SD)	8.63 (5.79)	8.81 (5.90)	0.577
Method of fertilization (*n*, %)			**< 0.001**
ICSI	4599 (79.6)	348 (100)	
IVF	1181 (20.4)	0 (0)	
Fertilization rate (mean, SD)	74.00 (24.39)	72.62 (23.88)	0.307
Number of embryos transferred (mean, SD)	2.10 (1.11)	2.01 (1.08)	0.162
Embryo quality (*n*, %)			
Cleavage			0.251
Good	5997 (61.8)	323 (58.7)	
Fair	2869 (29.6)	167 (30.4)	
Poor	648 (6.7)	45 (8.1)	
Unavailable	192 (2.0)	15 (2.7)	
Blastocyst			0.112
Excellent	501 (24.2)	38 (27.9)	
Good	563 (27.2)	38 (27.9)	
Average	616 (29.8)	28 (20.6)	
Poor	387 (18.7)	32 (23.5)	
Developmental stage at time of transfer (*n*, %)			0.231
Cleavage	3938 (70.9)	228 (67.9)	
Blastocyst	1615 (29.1)	108 (32.1)	

cleavage and $p=0.112$ for blastocyst) or for developmental stage of embryo at the time of transfer ($p=0.231$).

Cycle outcomes

Logistic regression models are displayed in Table 3, which displayed no significant associations for all outcomes of interest. The adjusted odds of live birth were 1.10 (95%CI 0.83–1.45) among those with sperm concentration of ≥ 5 million/ml and 1.27 (95%CI 0.77–2.09) among those with sperm concentration of < 5 million/ml. For secondary outcomes, the adjusted odds of a clinical IUP and miscarriage were 1.09 (95%CI 0.83–1.43) and 0.97 (95%CI 0.59–1.59), respectively, for those using sperm with concentration of ≥ 5 million/ml. For those using sperm with a concentration of < 5 million/ml, the odds of a clinical IUP were 1.17 (95%CI 0.72–1.90) and for miscarriage were 0.89 (95%CI 0.36–2.18). A sensitivity analysis was completed among only those with ICSI ($n=4947$), and similarly, no significant differences were observed for any outcomes of interest (Supplementary Table 1).

Discussion

Despite numerous reports examining the use of fresh versus frozen sperm, the majority of the literature has focused on specific clinical subgroups, namely men with infertility and/or subfertility. We demonstrate that in a large cohort of men with normal sperm concentration but also those with severe oligozoospermia, the use of fresh or frozen sperm has minimal impact on IVF outcomes during fresh autologous embryo transfer cycles.

In our series, there were no significant differences with respect to rates of fertilization. Sensitivity analyses were completed using variable thresholds for fertilization, but the difference remained insignificant at both 50% ($p=0.451$) and 75% ($p=0.553$) for fresh versus frozen ejaculated sperm, respectively. Other reports have suggested an overall fertilization benefit with the use of fresh sperm in heterogeneous groups, but when comparing a similar population (i.e., normospermic men), no significant differences were detected [9]. The same study also demonstrated similar implantation and pregnancy rates between cohorts using fresh and frozen sperm [9]. A recent study, in an attempt

Table 3 Multivariable logistic regression with odds ratios of pregnancy outcomes by maternal age and sperm concentration

	Frozen sperm	Fresh sperm
Sperm concentration (≥5 million/ml) ($n=5716$)		
Clinical IUP		
Proportion (n, %)	103/259 (39.8)	2284/5457 (41.9)
Unadjusted OR (95% CI)	*ref*	1.09 (0.85–1.41)
Adjusted OR (95% CI)		1.09 (0.83–1.43)
Miscarriage		
Proportion (n, %)	18/259 (6.9)	377/5457 (6.9)
Unadjusted OR (95% CI)	*ref*	0.99 (0.61–1.62)
Adjusted OR (95% CI)		0.97 (0.59–1.59)
Live birth		
Proportion (n, %)	85/259 (32.8)	1884/5457 (34.5)
Unadjusted OR (95% CI)	*ref*	1.08 (0.83–1.41)
Adjusted OR (95% CI)		1.10 (0.83–1.45)
Sperm concentration (<5 million/ml) ($n=412$)		
Clinical IUP		
Proportion (n, %)	40/89 (44.9)	159/323 (49.2)
Unadjusted OR (95% CI)	*ref*	1.19 (0.74–1.90)
Adjusted OR (95% CI)		1.17 (0.72–1.90)
Miscarriage		
Proportion (n, %)	7/89 (7.9)	22/323 (6.8)
Unadjusted OR (95% CI)	*ref*	0.86 (0.35–2.07)
Adjusted OR (95% CI)		0.89 (0.36–2.18)
Live birth		
Proportion (n, %)	32/89 (36.0)	136/323 (42.1)
Unadjusted OR (95% CI)	*ref*	1.30 (0.80–2.11)
Adjusted OR (95% CI)		1.27 (0.77–2.09)

*Adjusted for male age, female age, and sperm concentration

to control for oocyte quality, demonstrated that with paired recipients from the same donor, fresh sperm did result in superior live birth rates [16]. While this study does attempt to assess the isolated effect of frozen sperm, it is limited by its external generalizability.

While there remains some concern about the impacts of freezing on sperm motility and vitality, there is speculation that those sperm that survive the freeze–thaw cycle are alternatively more robust and of higher quality [4]. It may also be that the effects of freezing on sperm are more pronounced in men with infertility; that is, there is a smaller detrimental effect of freezing on men with normal semen quality, potentially explaining the minimal impact seen in our study [17].

In our series, men in the fresh sperm cohort did have greater sperm concentrations than those using frozen sperm. Men in the frozen group were also slightly older. While lower concentrations may be due to freezing and thawing of the sample, this finding may also suggest a selection bias of older men with poorer sperm quality in the frozen sperm group. If this group was considered at higher risk for poorer outcomes, this would bias our findings away from the null, reinforcing the absence of any impact on fresh over frozen sperm in our study.

These findings have significant implications for men who decide to or require freezing of their semen sample for use in ART. In our series, the majority of IVF was performed with ICSI, including all of the frozen sperm samples. This is important as the discovery of ICSI has been paramount to overcoming numerous barriers to assisted reproduction, including suboptimal semen parameters, and when considered in the context of both sperm sorting and newer technologies (i.e., microfluidics), we have become more capable of choosing more optimal sperm regardless of the source [18–21]. Therefore, certain key patient populations may benefit more than others, such as those who are seeking fertility preservation and who may not recover spermatogenesis after a disease treatment or for social reasons and partner availability when women are undergoing IVF.

One limitation of our study includes its retrospective design, however, the data arising from the largest sample size, to our knowledge, addressed this question over a substantial time period. We did not examine the impact of freezing on semen parameters or if there was any resultant DNA damage. Our study has a larger portion of ICSI than other centers, which may limit its external validity. Furthermore, our study only utilized fresh embryo transfer cycles, which may portend a patient population with a different prognosis than those who undergo upfront embryo cryopreservation, which may also limit its generalizability.

Conclusion

The use of fresh or frozen ejaculated sperm has no impact on reproductive outcomes in fresh ET cycles.

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Declarations

Conflict of interest The authors declare no competing interests.

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