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Research Article

Keywords: donor sperm, cryopreservation, density gradient centrifugation, sperm motility, IVF clinical pregnancy

Posted Date: November 9th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11298236/v1

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Additional Declarations: No competing interests reported.

Predictive value of semen parameters across three sequential processing stages for clinical pregnancy in donor sperm IVF cycles

Running title: Semen parameter predictive value in IVF

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Abstract

Background: Conventional semen parameters exhibit limited predictive value for in vitro fertilization (IVF) outcomes, and their performance across sequential sperm processing stages remains poorly characterized in donor sperm cycles. This study aimed to evaluate whether semen parameters measured at pre-cryopreservation, post-thaw, and post-density gradient centrifugation (DGC) stages can predict clinical pregnancy in IVF cycles using cryopreserved donor sperm.

Methods: We conducted a single-center retrospective cohort study at the CITIC Xiangya Sperm Bank, Reproductive & Genetic Hospital of CITIC-Xiangya. A total of 775 first IVF oocyte retrieval cycles using donor sperm (September 2022–January 2025) were included and stratified into clinical pregnancy (n = 357) and non-pregnancy (n = 418) groups. Non-parametric tests, Spearman correlation, univariate and multivariate logistic regression, and receiver operating characteristic (ROC) curve analyses were performed to assess group differences and predictive performance.

Results: All semen parameters changed significantly across cryopreservation and DGC (all $P < .001$). Only post-thaw total motility was slightly higher in pregnant cycles (45.00% vs 44.45%, $P = .036$) and remained an independent predictor after adjustment (adjusted OR = 1.023, 95% CI 1.004–1.043, $P = .020$). All individual and combined conventional semen parameters showed poor discriminative capacity for clinical pregnancy, with AUC values ranging from 0.50 to 0.55.

Conclusions: Post-thaw total motility can serve as a simple quality control marker for donor sperm allocation. Standard semen metrics across all processing stages lack sufficient power to forecast IVF pregnancy outcomes, indicating that novel sperm functional biomarkers are required in clinical practice.

Keywords: donor sperm; cryopreservation; density gradient centrifugation; sperm motility; IVF clinical pregnancy

Introduction

Infertility affects approximately 17.5% of the global adult population, corresponding to roughly one in six individuals worldwide, according to the latest World Health Organization (WHO) report [1]. Assisted reproductive technology (ART) has become an essential treatment for infertile couples, with *in vitro* fertilization (IVF) representing one of its most widely used approaches [2]. The success of IVF is influenced by multiple factors, including female age, ovarian reserve, endometrial receptivity, embryo quality, and male reproductive factors [3,4]. Among these, semen quality is an important determinant of male fertility. However, establishing clinically meaningful thresholds for conventional semen parameters in relation to pregnancy outcomes has remained challenging. Although several studies have reported associations between specific semen characteristics and ART success rates [5,6], a recent meta-analysis found no significant association between sperm concentration, motility, or morphology and clinical pregnancy rates [7]. Likewise, conventional semen analysis alone cannot reliably predict the likelihood of pregnancy for an individual couple, except in the most extreme clinical circumstances [8].

Most previous studies have evaluated semen parameters at a single stage of the ART process, without considering changes that occur throughout sperm processing. In clinical practice, not all patients undergoing ART use fresh semen; many require cryopreserved sperm, and the freeze–thaw process is known to impair sperm motility and function [9]. Furthermore, regardless of whether fresh or frozen-thawed semen is used, sperm samples typically undergo laboratory preparation before insemination, most commonly by swim-up or density gradient centrifugation (DGC). These procedures enrich sperm populations with superior motility and morphology and increase the proportion of motile spermatozoa [10]. Consequently, semen characteristics can differ substantially between fresh, post-thaw, and processed samples. Assessment of semen parameters after cryopreservation and laboratory preparation may therefore provide more clinically relevant information than evaluation of fresh semen alone [11].

To date, however, no study has comprehensively examined the relationship between semen parameters measured at different stages of sperm processing and clinical pregnancy outcomes in donor sperm IVF cycles. Therefore, the present study aimed to evaluate the predictive value of semen parameters assessed at three sequential stages (pre-cryopreservation, post-thaw, and post-density gradient centrifugation) for clinical pregnancy following donor sperm IVF. To address this question, we performed a retrospective analysis of semen characteristics and pregnancy outcomes from donor sperm IVF cycles conducted through the CITIC Xiangya Sperm Bank between 2022 and 2025.

Materials and methods

Study population and participants

A retrospective review was conducted using semen analysis records from men applying for sperm donation at the Reproductive and Genetic Hospital of CITIC-Xiangya between September 2022 and January 2025. From this database, we identified all fresh oocyte retrieval cycles in which donor sperm was used for IVF-ET. A total of 1,365 cycles were initially screened, and 775 eligible cycles were ultimately included in the analysis (Figure 1). Only first oocyte retrieval cycles undergoing IVF were eligible for inclusion. In addition, semen samples had to be prepared exclusively using DGC. Cycles were excluded if they had incomplete semen analysis data, including missing key pre- or post-processing parameters (e.g. sperm concentration or motility); incomplete embryological data, including unrecorded fertilization or cleavage outcomes; or unavailable pregnancy outcome data, including the absence of pregnancy testing after embryo transfer or loss to follow-up before confirmation of clinical pregnancy.

Semen analysis

All semen samples were evaluated in accordance with the WHO 2010 laboratory manual for the examination and processing of human semen [12]. After complete liquefaction and within 1 h of ejaculation, semen volume, sperm concentration, and

sperm motility were assessed. Sperm motility was classified according to the WHO four-category grading system as grade A (rapid progressive motility), grade B (slow progressive motility), grade C (non-progressive motility), and grade D (immotile spermatozoa) [12].

Criteria for screening sperm donors in China

All sperm donors included in this study were recruited and screened in accordance with the "Basic Standards and Technical Specifications for Human Sperm Banks" issued by the Ministry of Health of the People's Republic of China in 2003 [13]. Eligible donors were men aged 22–45 years who underwent standardized physical and psychological assessments conducted by qualified clinicians. Individuals with a family history of hereditary disease or belonging to groups at high risk of sexually transmitted infections were excluded.

Eligible candidates subsequently provided fresh semen samples for quality assessment. Samples were accepted for cryopreservation only if they met all of the following criteria: liquefaction time <60 min, sperm concentration ≥60 × 10^6/mL, progressive motility ≥50%, and normal sperm morphology >30%. To ensure post-thaw quality, one cryovial from each batch was thawed and evaluated. Samples were required to demonstrate total motility ≥40%, a total progressive motile sperm count ≥12 × 10^6 per cryovial, and a freeze–thaw survival rate ≥60%. Samples that failed to meet any of these criteria were discarded and were not used for clinical treatment.

All potential donors also underwent comprehensive laboratory screening. Donors were required to test negative for human immunodeficiency virus (HIV) types 1 and 2, hepatitis B virus, hepatitis C virus, *Treponema pallidum*, *Neisseria gonorrhoeae*, *Mycoplasma* spp., *Chlamydia* spp., cytomegalovirus, *Toxoplasma gondii*, rubella virus, and herpes simplex virus types 1 and 2. In addition, all donors were required to have a normal chromosomal karyotype. To minimize the risk of HIV transmission during the serological window period, all cryopreserved semen samples underwent a mandatory 6-month quarantine. Samples were released for clinical use only after repeat HIV testing confirmed a negative result at the end of the quarantine period.

Sperm cryopreservation and thawing

Fresh semen samples were mixed with test yolk buffer freezing medium to a final concentration of 25% (v/v), equilibrated at room temperature for 15 min, and aliquoted into 1.8-mL cryogenic straws (Thermo Fisher Scientific, Waltham, MA, USA). Samples were cooled using a programmable controlled-rate freezer (Thermo7451; Thermo Fisher Scientific) and subsequently stored in liquid nitrogen. To assess post-thaw quality before clinical use, one straw from each frozen sample was retrieved 30 min after cryostorage and thawed in a 37°C water bath for 15 min. Post-thaw sperm motility was then evaluated according to the sperm bank quality-control protocol. Throughout the study period, donor screening criteria, cryopreservation procedures, laboratory equipment, and freezing medium formulations remained unchanged to ensure methodological consistency.

Density gradient centrifugation

Sperm preparation was performed using DGC with 45% and 90% ISolate density gradient medium (Irvine Scientific, Santa Ana, CA, USA). Up to 2 mL of liquefied semen was carefully layered onto the discontinuous gradient and centrifuged at 500 × g for 20 min. The resulting sperm pellet was resuspended in IVF-100 culture medium (Vitrolife, Gothenburg, Sweden) and centrifuged again at 500 × g to remove residual gradient medium. After washing, the final sperm suspension was prepared in fresh IVF-100 (Vitrolife) and incubated at 37°C in an atmosphere of 5% CO_2 until insemination.

Statistical analysis

Participants were classified into either the clinical pregnancy group or the non-pregnancy group according to pregnancy outcome. Clinical pregnancy was defined as the presence of an intrauterine gestational sac detected by transvaginal ultrasonography 5–6 weeks after embryo transfer. Semen parameters were evaluated at three sequential processing stages: pre-cryopreservation, post-thaw, and post-DGC. Because all semen parameters and embryological variables exhibited non-normal distributions, non-parametric statistical methods were used throughout the analysis. Changes in semen parameters across the three processing stages were assessed using the Wilcoxon signed-rank test. Differences in semen parameters and IVF laboratory

outcomes between the clinical pregnancy and non-pregnancy groups were compared using the Mann–Whitney U test. Associations between semen parameters and fertilization and cleavage rates were evaluated using Spearman rank correlation analysis. Univariable and multivariable logistic regression analyses were performed to examine the associations between semen parameters and clinical pregnancy. Receiver operating characteristic (ROC) curve analysis was used to evaluate the predictive performance of individual semen parameters and parameter combinations for clinical pregnancy. Statistical analyses were conducted using SPSS version 27.0 (IBM Corp., Armonk, NY, USA) and MedCalc version 20 (MedCalc Software Ltd., Ostend, Belgium). All tests were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Dynamic changes in semen parameters across processing stages

All semen parameters changed significantly following cryopreservation and DGC (all $P < 0.001$). Median sperm concentration decreased from 64.0 × 10^6/mL in fresh samples to 52.0 × 10^6/mL after thawing and further declined to 21.0 × 10^6/mL after DGC. Progressive motility (A+B) decreased from 50.0% in fresh semen to 29.2% after thawing but increased markedly to 92.3% following DGC. Similarly, total motility (A+B+C) increased from 44.8% after thawing to 95.5% after DGC (Supplementary Table 1).

Parameter comparison by pregnancy outcomes

As shown in Table 1, no significant differences were observed in pre-cryopreservation sperm concentration or progressive motility between the clinical pregnancy and non-pregnancy groups ($P = 0.801$ and $P = 0.931$, respectively). Similarly, after thawing, sperm concentration and progressive motility remained comparable between the two groups ($P = 0.596$ and $P = 0.231$, respectively). The only post-thaw semen parameter that differed significantly was total motility (A+B+C), which was slightly higher in the clinical pregnancy group than in the non-pregnancy group (45.00% versus 44.45%, $P = 0.036$). Following DGC, progressive motility exceeded 92% and

total motility exceeded 95% in both groups. No significant differences were observed in any post-DGC semen parameter, including total motility (95.50% versus 95.50%, $P = 0.254$). With respect to embryological outcomes, both the fertilization rate (0.88 versus 0.86, $P = 0.004$) and cleavage rate (0.86 versus 0.83, $P = 0.002$) were significantly higher in the clinical pregnancy group than in the non-pregnancy group. However, the numbers of retrieved oocytes, fertilized oocytes, and cleaved embryos did not differ significantly between the groups.

Correlation between semen parameters and embryo development indicators

As shown in Table 2, pre-cryopreservation sperm concentration was weakly positively correlated with cleavage rate ($r = 0.072$, $P = 0.046$), whereas post-thaw progressive motility was weakly negatively correlated with cleavage rate ($r = -0.072$, $P = 0.046$). Although these correlations reached statistical significance, their magnitudes were negligible ($|r| < 0.1$) and therefore unlikely to be clinically meaningful. No significant correlations were identified between any other semen parameter and either fertilization rate or cleavage rate at any processing stage (all $P > 0.05$).

Univariable and multivariable logistic regression analyses

As shown in Table 3, univariable logistic regression analysis identified post-thaw total motility (A+B+C) as the only semen parameter significantly associated with clinical pregnancy (OR = 1.021, 95% CI 1.002–1.040, $P = 0.029$). No other semen parameter measured at any processing stage was significantly associated with clinical pregnancy (all $P > 0.05$). Among the embryological variables, both fertilization rate (OR = 3.967, 95% CI 1.731–9.093, $P < 0.001$) and cleavage rate (OR = 4.262, 95% CI 1.922–9.455, $P < 0.001$) were significantly associated with clinical pregnancy in the univariable analyses, suggesting that early embryo developmental competence may be closely related to subsequent pregnancy outcomes.

Variables with $P < 0.10$ in the univariable analyses, together with clinically relevant covariates including the number of retrieved oocytes, fertilization rate, and cleavage rate, were entered into the multivariable logistic regression model. After adjustment, post-thaw total motility remained independently associated with clinical pregnancy (adjusted OR = 1.023, 95% CI 1.004–1.043, $P = 0.020$). Specifically, each

1-percentage-point increase in post-thaw total motility was associated with a 2.3% increase in the odds of clinical pregnancy.

Diagnostic performance of indicators for clinical pregnancy outcomes

ROC curve analysis was performed to evaluate the ability of semen parameters measured at the three processing stages to discriminate between cycles that resulted in clinical pregnancy and those that did not. Figure 2 presents the superimposed ROC curves for all individual and combined conventional semen parameters. Overall, the discriminative performance of these parameters was poor (Table 4). For pre-cryopreservation semen parameters, the AUCs were 0.51 for sperm concentration and 0.50 for progressive motility, indicating no meaningful predictive value. Similarly, among the post-thaw parameters, total motility was the only variable that differed significantly between the pregnancy groups; however, its predictive performance remained limited, with an AUC of 0.54. The corresponding AUCs for post-thaw sperm concentration and progressive motility were 0.51 and 0.50, respectively. The combination of post-thaw sperm concentration and total motility yielded the highest AUC observed in the study, although the value remained low at 0.55 (95% CI 0.52–0.59). Despite the substantial improvement in semen quality following DGC, the predictive performance of post-DGC parameters remained poor, with AUC values ranging from 0.51 to 0.53. Furthermore, DeLong tests demonstrated no significant differences in AUC among the three processing stages for any individual parameter or parameter combination (all $P > 0.05$). These findings indicate that conventional semen parameters, whether assessed individually or in combination, have limited ability to predict clinical pregnancy outcomes in donor sperm IVF cycles.

Discussion

Semen quality assessment plays an important role in both the evaluation of male infertility and the selection of appropriate ART strategies, including IVF and ICSI [14,15]. However, the relationship between conventional semen parameters and IVF outcomes remains controversial [8,16]. In the present study, post-thaw total motility was

slightly but significantly higher in cycles that resulted in clinical pregnancy than in those that did not (45.00% versus 44.45%, $P = 0.036$). After adjustment for potential confounders, including the number of retrieved oocytes, fertilization rate, and cleavage rate, post-thaw total motility remained independently associated with clinical pregnancy (adjusted OR = 1.023, 95% CI 1.004–1.043, $P = 0.020$). Specifically, each 1-percentage-point increase in post-thaw total motility was associated with a 2.3% increase in the odds of clinical pregnancy. These findings suggest that post-thaw total motility may provide clinically relevant information regarding reproductive potential in donor sperm IVF cycles. Our results are consistent with previous studies reporting associations between post-thaw sperm quality and reproductive outcomes. In a large retrospective study of 3,247 donor sperm IUI cycles, post-thaw total motility was identified as an independent predictor of ongoing pregnancy [17]. Similarly, earlier studies of donor insemination demonstrated that post-thaw motile sperm concentration was significantly associated with pregnancy rates and represented an important determinant of treatment success [18]. Experimental evidence from a goat IVF model also showed that post-thaw sperm motility reliably reflected fertilization potential [19]. Although the observed effect size was modest, even small differences in post-thaw motility may be relevant in large-scale donor sperm programmes, where sperm quality assessment is a critical component of sample selection and allocation. Nevertheless, the limited magnitude of the association should be interpreted cautiously, particularly in light of the poor discriminative performance observed in the ROC analyses. Cryopreservation can induce structural and functional damage to spermatozoa, including reduced motility, DNA fragmentation, and acrosomal injury, thereby compromising fertilization capacity [20,21]. In addition, DGC is the most widely used sperm preparation method in clinical ART and is designed to enrich sperm populations with superior motility and quality [22,23]. While this selection process improves overall semen quality, it may also reduce the variability between samples by preferentially retaining the most motile spermatozoa. As a result, post-DGC semen parameters may be less capable of distinguishing differences in reproductive potential among donor sperm samples. This observation may help explain why post-thaw total

motility, rather than pre-cryopreservation or post-DGC parameters, was associated with clinical pregnancy in the present study. Post-thaw motility reflects a sperm sample's ability to withstand cryopreservation-induced stress and may therefore provide additional information regarding sperm functional competence. Consequently, post-thaw total motility may represent a useful indicator for donor sperm quality assessment in sperm banks. However, this interpretation should be viewed with caution and requires confirmation in independent studies. Not all previous investigations have reached similar conclusions. Several studies have reported no significant associations between conventional semen parameters, including sperm concentration, motility, and morphology, and ART pregnancy outcomes [7,16]. It should be noted, however, that most of these studies evaluated semen characteristics at only a single processing stage and did not consider changes occurring throughout the sperm processing workflow. This methodological difference may partly explain the discrepancy between their findings and those of the present study.

Fertilization rate and cleavage rate are widely recognized indicators of early embryo developmental competence. Fertilization rate has been identified as an independent predictor of implantation, whereas early cleavage is considered a robust marker of embryo quality and a valuable predictor of IVF/ICSI outcomes [24,25,26,27]. Consistent with these observations, both fertilization rate (0.88 versus 0.86, $P = 0.004$) and cleavage rate (0.86 versus 0.83, $P = 0.002$) were significantly higher in the clinical pregnancy group than in the non-pregnancy group in the present study. Furthermore, univariable logistic regression analyses demonstrated significant associations between clinical pregnancy and both fertilization rate (OR = 3.967, 95% CI 1.731–9.093, $P < 0.001$) and cleavage rate (OR = 4.262, 95% CI 1.922–9.455, $P < 0.001$). These findings are in agreement with a large registry study of 15,803 first fresh IVF cycles, which reported that the number of fertilized oocytes was an independent predictor of both clinical pregnancy and live birth [28]. Notably, however, no meaningful correlations were observed between semen parameters measured at any processing stage and either fertilization rate or cleavage rate. This observation is consistent with previous studies showing that conventional semen parameters alone cannot reliably

predict fertilization or blastocyst development and that additional functional markers, such as acrosomal status and chromatin integrity, may be required to more accurately assess sperm fertilizing capacity [29]. These findings suggest that conventional semen parameters provide only limited information regarding the functional competence of spermatozoa and their contribution to subsequent embryo development. This may partly explain their limited value for predicting clinical pregnancy outcomes in donor sperm IVF cycles.

Despite the significant association between post-thaw total motility and clinical pregnancy, ROC analysis demonstrated that none of the conventional semen parameters assessed at the three processing stages, whether evaluated individually or in combination, showed meaningful discriminative ability for predicting clinical pregnancy. This finding is consistent with previous reports. For example, models based solely on conventional semen parameters achieved AUC values of only 0.57–0.58 for predicting total fertilization failure in conventional IVF cycles, indicating limited predictive performance [30]. Similarly, a study of 796 IVF cycles reported that routine semen parameters were not reliable predictors of pregnancy outcomes [31]. In donor sperm populations, the initial total motile sperm count and post-processing motility yielded AUC values of 0.602 and 0.595, respectively, which, although statistically significant, were considered to have limited clinical utility [32]. The findings suggest that conventional semen parameters alone provide insufficient accuracy for predicting IVF pregnancy outcomes, regardless of the stage at which they are assessed.

To our knowledge, this is the first study to comprehensively evaluate semen parameters throughout the entire donor sperm processing workflow, from pre-cryopreservation assessment to post-thaw and post-DGC evaluation, in relation to clinical pregnancy outcomes. Nevertheless, several limitations should be acknowledged. First, information on female recipient age was unavailable because of the anonymous donor-assisted reproduction policy in China. This is an important limitation, as maternal age is a well-established determinant of IVF success. Second, the study population consisted exclusively of highly selected sperm donors with

semen quality substantially above that of the general male population, which may limit the generalizability of the findings. Third, the retrospective single-centre design may introduce selection bias and limits the ability to establish causal relationships. Consequently, the findings require validation in larger, prospective, multicentre studies. Future research should also investigate whether advanced sperm function markers, including DNA fragmentation and chromatin integrity, provide additional predictive value beyond conventional semen parameters in donor sperm IVF cycles.

Conclusion

This is the first study to evaluate the relationship between semen parameters measured across three sequential processing stages and clinical pregnancy outcomes in donor sperm IVF cycles. Post-thaw total motility was independently associated with clinical pregnancy and may represent a useful indicator of donor sperm quality. However, despite this association, conventional semen parameters assessed before cryopreservation, after thawing, and after DGC demonstrated limited ability to predict clinical pregnancy outcomes. These findings suggest that post-thaw semen quality may provide additional information for donor sperm assessment, but conventional semen parameters alone are insufficient for accurate prediction of IVF success. Therefore, semen parameters should be interpreted with caution when counseling patients or estimating the likelihood of clinical pregnancy. Future studies are warranted to determine whether advanced sperm function markers can improve outcome prediction in donor sperm IVF cycles.

Table 1. Comparison of semen parameters and embryological outcomes between the clinical pregnancy and non-pregnancy groups.

Parameter	Non-pregnancy (n = 418)	Pregnancy group (n = 357)	*P* value
Pre-cryopreservation sperm parameters			
Pre-cryopreservation concentration ($\times 10^6$/mL)	62.00 (60.00, 70.00)	64.00 (60.00, 70.00)	0.801
Pre-cryopreservation progressive motility (A+B, %)	50.00 (50.00, 51.50)	50.00 (50.00, 51.50)	0.931
Post-thaw sperm parameters			
Post-thaw concentration ($\times 10^6$/mL)	52.00 (42.00, 61.00)	52.00 (42.00, 62.00)	0.596
Grade A (rapid progressive, %)	8.60 (6.70, 11.60)	8.70 (6.90, 12.20)	0.230
Grade B (slow progressive, %)	20.00 (17.20, 22.70)	20.00 (17.70, 22.90)	0.352
Grade C (non-progressive, %)	12.50 (10.80, 15.00)	12.80 (10.90, 15.70)	0.304
Progressive motility (A+B, %)	29.10 (24.50, 35.00)	29.70 (25.00, 35.00)	0.231
Total motility (A+B+C, %)	44.45 (38.10, 48.00)	45.00 (39.50, 50.00)	0.036
Post-DGC sperm parameters			
Post-DGC concentration ($\times 10^6$/mL)	21.00 (15.00, 22.00)	21.00 (15.00, 22.00)	0.806
Grade A (rapid progressive, %)	36.40 (33.30, 41.20)	36.40 (33.30, 41.20)	0.947
Grade B (slow progressive, %)	54.50 (52.20, 59.10)	54.50 (50.00, 59.10)	0.886
Grade C (non-progressive, %)	2.40 (1.90, 4.50)	2.40 (2.00, 4.60)	0.353
Progressive motility (grades A+B, %)	92.30 (90.90, 95.20)	92.30 (90.90, 95.20)	0.731
Total motility (grades A+B+C, %)	95.50 (93.80, 96.70)	95.50 (93.80, 97.10)	0.254
IVF laboratory outcomes			
Number of retrieved oocytes	12.00 (8.00, 17.00)	13.00 (9.00, 15.00)	0.899
Number of fertilized oocytes	9.00 (6.00, 14.00)	10.00 (7.00, 13.00)	0.161
Number of cleaved embryos	9.00 (6.00, 13.00)	10.00 (7.00, 13.00)	0.078
Fertilization rate (%)	0.86 (0.72, 0.95)	0.88 (0.77, 1.00)	0.004
Cleavage rate (%)	0.83 (0.68, 0.93)	0.86 (0.75, 0.95)	0.002

Data are presented as median (interquartile range). *P*-values were calculated using the Mann–Whitney U test. DGC, density gradient centrifugation. Fertilization and cleavage rates are expressed as proportions.

Table 2. Spearman correlations between semen parameters and embryological outcomes.

Indicator	Fertilization Rate		Cleavage Rate	
	r	*P*	*r*	*P*
Pre-cryopreservation concentration	0.048	0.183	0.072	0.046
Pre-cryopreservation progressive motility (A+B)	0.032	0.367	0.036	0.315
Post-thaw Concentration	0.020	0.581	0.029	0.417
Post-thaw progressive motility (A+B)	−0.069	0.056	−0.072	0.046
Post-thaw total motility (A+B+C)	−0.023	0.517	−0.028	0.433
Post-DGC concentration	0.011	0.752	0.012	0.729
Post-DGC motility (A+B)	−0.036	0.317	−0.040	0.270
Post-DGC total motility (A+B+C)	−0.042	0.239	−0.048	0.184

DGC, density gradient centrifugation. Correlations were assessed using Spearman rank correlation analysis.

Table 3. Univariable and multivariable logistic regression analyses for clinical pregnancy.

Indicator	Univariate analysis OR (95% CI)	*P*	Multivariate analysis OR (95% CI)	*P*
Pre-cryopreservation concentration ($\times 10^6$/mL)	0.994 (0.979–1.009)	0.428	—	—
Pre-cryopreservation progressive motility (A+B)	0.973 (0.888–1.067)	0.562	—	—
Post-thaw concentration ($\times 10^6$/mL)	1.003 (0.991–1.015)	0.658	—	—
Post-thaw progressive motility (A+B)	1.016 (0.995–1.037)	0.133	—	—
Post-thaw total motility (A+B+C)	1.021 (1.002–1.040)	0.029	1.023 (1.004–1.043)	0.020
Post-DGC concentration ($\times 10^6$/mL)	1.005 (0.965–1.046)	0.809	—	—
Post-DGC motility (A+B)	1.014 (0.974–1.056)	0.495	—	—
Post-DGC total motility (A+B+C)	1.046 (0.984–1.113)	0.151	—	—
Number of cleaved embryos	0.992 (0.968–1.018)	0.556	0.993 (0.967–1.018)	0.567
Fertilization rate (%)	3.967 (1.731–9.093)	<0.001	0.373 (0.018–7.721)	0.524
Cleavage rate (%)	4.262 (1.922–9.455)	<0.001	10.812 (0.589–198.610)	0.109

Note: The multivariable model included variables with $P < 0.10$ in the univariable analyses together with clinically relevant covariates. Multicollinearity was assessed using variance inflation factors (VIFs), and all VIF values were <2.0, indicating no evidence of problematic multicollinearity. $P < 0.05$ was considered statistically significant.

Table 4. Diagnostic performance of semen parameters at different processing stages for predicting clinical pregnancy.

Parameter	Pre-cryopreservation	Post-thaw	*P* [a]	Post-DGC	*P* [b]
Sperm concentration (10^6/mL)					
Sensitivity (%)	52.94 [47.6, 58.2] (189/357)	19.89 [15.9, 24.4] (71/357)	<.01	71.71 [66.7, 76.3] (256/357)	<.01
Specificity (%)	51.44 [46.5, 56.3] (215/418)	84.69 [80.9, 88.0] (354/418)	<.01	30.14 [25.8, 34.8] (126/418)	<.01
AUC	0.51 [0.47, 0.54]	0.51 [0.48, 0.55]	.83	0.51 [0.47, 0.54]	.77
Progressive motility (A+B, %)					
Sensitivity (%)	5.60 [3.5, 8.5] (20/357)	88.8 [85.1, 91.9] (317/357)	<.01	44.54 [39.3,49.9] (159/357)	<.01
Specificity (%)	91.15 [88.0, 93.7] (381/418)	16.75 [13.1, 20.7] (70/418)	<.01	58.61 [53.7, 63.4] (245/357)	<.01
AUC	0.50 [0.47, 0.54]	0.53 [0.49, 0.56]	.40	0.51 [0.47, 0.54]	.43
Combination of concentration and motility (A+B)					
Sensitivity (%)	87.11 [83.22, 90.4] (311/357)	62.18 [56.9, 67.2] (222/357)	<.01	75.35 [70.5, 79.7] (269/357)	<.01
Specificity (%)	17.70 [14.2, 21.7] (74/418)	45.45 [40.6, 50.4] (190/418)	<.01	27.99 [23.7, 32.6] (117/418)	<.01
AUC	0.50 [0.47, 0.54]	0.54 [0.51, 0.58]	.22	0.51 [0.48, 0.55]	.24
Total motility (A+B+C, %)					
Sensitivity (%)	—	33.61 [28.7, 38.8] (120/357)	—	64.43 [59.2, 69.4] (230/357)	<.01
Specificity (%)	—	75.84 [71.4, 79.9] (317/418)	—	40.43 [35.7, 45.3] (169/418)	<.01
AUC	—	0.54 [0.51, 0.58]	—	0.52 [0.49, 0.56]	.45
Combination of concentration and motility(A+B+C)					
Sensitivity (%)	—	26.33 [21.8, 31.2] (94/357)	—	60.78 [55.5, 65.9] (217/357)	<.01
Specificity (%)	—	82.78 [78.8, 86.3] (346/418)	—	44.98 [40.1, 49.9] (188/418)	<.01
AUC	—	0.55 [0.52, 0.59]	—	0.53 [0.49, 0.56]	.38

[a]Comparison with the pre-cryopreservation group.

[b]Comparison with the post-thaw group.

AUC, area under the receiver operating characteristic curve; DGC, density gradient centrifugation.

Values in brackets represent 95% confidence intervals

Supplementary Table 1. Pairwise comparisons of semen parameters across consecutive processing stages.

Figure legends

Figure 1. Flowchart of inclusion and exclusion for donor sperm IVF cycles.

Figure 2. ROC curves of semen parameters for predicting clinical pregnancy.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Hunan Normal University (approval number: MLTC-2026-350). Written informed consent was obtained from all sperm donors at the time of donation for the use of their semen samples and de-identified clinical data in this research.

Clinical trial number

Not applicable.

Consent for publication

Not applicable. All data used in this study are de-identified aggregated data, and no individually identifiable donor information is included.

Availability of data and materials

The de-identified aggregated datasets supporting the conclusions of this article are available from the corresponding author on reasonable request. The full raw clinical dataset cannot be shared publicly due to donor privacy regulations and institutional policy.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This work was supported by Funding from National Clinical Research Center for Obstetrics and Gynecology (Peking University Third Hospital) [grant No. BYSYSZKF2021005], the Natural Science Foundation of Hunan Province [grant No. 2024JJ6725], the Research Foundation of Reproductive & Genetic Hospital of CITIC-Xiangya [grant No. YNXM-202205], Hunan Provincial College Students' Innovation and Entrepreneurship Training Program [grant No. S202510542459], and University-level College Students' Innovation and Entrepreneurship Training Program [grant No. 2025466]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions

Ji-Liang Tang: Formal analysis, Writing – original draft.

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Min-Hui Xu: Data curation.

Yu Chen: Investigation.

Jian Wu: Resources.

Li-Qing Fan: Resources.

Wen-Bing Zhu: Supervision.

Ke Tao: Conceptualization, Writing – review & editing, Supervision.

Chuan Huang: Conceptualization, Funding acquisition, Writing – review & editing, Supervision.

Acknowledgements

Not applicable.

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Figure 1. *Flowchart illustrating the inclusion and exclusion process for donor sperm IVF cycles included in the study. A total of 1,365 cycles were initially screened, and 775 eligible cycles were included in the final analysis.*

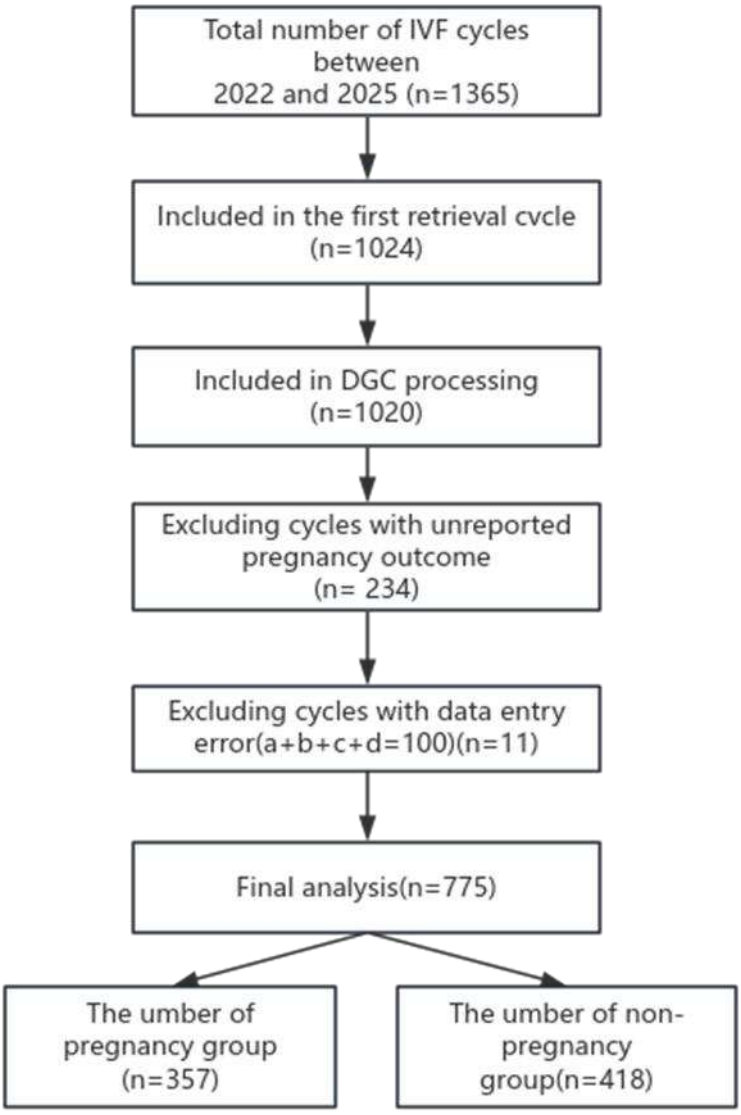
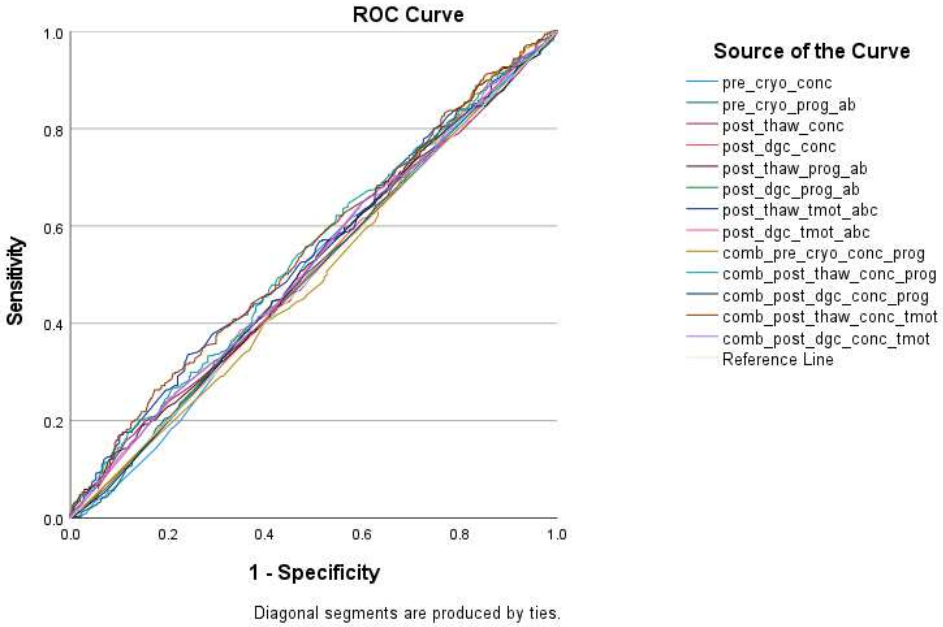


Figure 2. *Receiver operating characteristic (ROC) curves showing the performance of conventional semen parameters measured at different processing stages for predicting clinical pregnancy in donor sperm IVF cycles.*



Supplementary Files

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- SupplementaryTable1.docx