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Original Research Article

Comparison of reproductive outcomes in ICSI cycles after sperm selection techniques in advanced paternal age

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ABSTRACT

Background: Advanced paternal age is associated with declining semen parameters, increased sperm DNA fragmentation (SDF), and oxidative stress, which may negatively affect ART outcomes. Microfluidic sperm sorting has been proposed as a method to reduce SDF and improve sperm quality compared to conventional density gradient centrifugation.

Methods: Single-center retrospective cohort study including 193 ICSI cycles performed between January 2023 and December 2024. Couples were stratified by male age (<40 years vs. ≥40 years) and further subdivided by sperm preparation method (density gradient vs. microfluidics), forming four subgroups (A1: <40/DG, n=56; A2: <40/MF, n=59; B1: ≥40/DG, n=33; B2: ≥40/MF, n=45). Inclusion criteria: ≥10 mature oocytes, ICSI, frozen double blastocyst transfer, and sperm concentration ≥5×10^6/ml. Exclusion criteria: severe male factor infertility or donor gametes. Primary outcome: live birth rate. Secondary outcomes: clinical pregnancy, miscarriage, fertilization, and blastulation rates.

Results: No significant differences were observed in clinical pregnancy, miscarriage, or live birth rates between preparation methods within either age group (all p>0.05). Fertilization and blastulation rates were comparable across subgroups. A modest reduction in oocyte yield was noted in older males undergoing microfluidic preparation (A2 vs. B2; p=0.034), likely reflecting female- or cycle-specific factors. Demographic/clinical data are detailed in tables in the results section. Multivariate logistic regression showed that female age (OR 1.02, 95% CI 0.96–1.09, p=0.50) and subgroup membership were not significant predictors of clinical pregnancy. Pairwise comparisons with Tukey adjustment confirmed no significant differences between subgroups (e.g., A1 vs. B1: OR 1.91, p=0.515).

conclusion: Male age ≥40 years and sperm preparation method (microfluidics vs. density gradient) were not independent determinants of ART success in this cohort. Clinical decision-making regarding sperm selection should not be guided solely by paternal age or subgroup classification.

Keywords: Male infertility, Advanced paternal age, Microfluidics, Sperm processing, ICSI, Clinical pregnancy

INTRODUCTION

Reproductive success has traditionally been viewed through the lens of maternal factors, particularly age, which is well known to influence fertility, pregnancy outcomes, and the risk of chromosomal abnormalities such as aneuploidy. However, increasing attention has been directed toward the role of paternal factors, especially advanced paternal age (APA), in shaping reproductive outcomes. Although male reproductive function does not undergo a decline comparable to menopause in females, accumulating evidence suggests that aging in men is associated with gradual but significant changes in sperm quality and genetic integrity.[1-3] These alterations may adversely affect fertilization, embryo development, pregnancy outcomes, and even the long-term health of offspring.[1,4] Globally, paternal age has increased, with men >40 years showing 20–30% lower fertility and higher

SDF, which rises after 35 years and can increase 4- to 5-fold after age 50. In India, male factors account for nearly 50% of infertility, with a growing trend of delayed fatherhood (≥35 years) and increased prevalence of poor sperm parameters and DNA damage in older men. Oxidative stress (OS) is considered a major mechanistic pathway underlying sperm aging. Excess reactive oxygen species (ROS) can induce DNA strand breaks, lipid peroxidation, and protein oxidation, thereby impairing sperm function and embryo competence.[5,6]

Furthermore, conventional sperm preparation techniques involving repeated centrifugation may exacerbate ROS exposure and contribute to iatrogenic sperm damage. Consequently, alternative sperm selection strategies such as microfluidic sperm sorting have gained attention because of their ability to isolate sperm populations with lower DNA fragmentation and reduced oxidative injury.[7]

Despite these theoretical considerations, the clinical benefit of microfluidics over density gradient centrifugation in the context of APA remains unclear. Therefore, this study aimed to compare reproductive outcomes after density gradient versus microfluidic sperm selection in ICSI cycles for couples with advanced paternal age.

METHODS

Study type

This was a retrospective cohort study.

Study place

The study was conducted at Nova IVF Fertility, Kolkata, West Bengal, India.

Study duration

The study period was from January 2023 to December 2024.

Selection criteria of the patients

A total of 193 ICSI cycles were included.

Inclusion criteria

Couples with at least 10–14 mature oocytes retrieved at pickup, oocyte insemination by ICSI, frozen double embryo transfer at the blastocyst stage, and sperm concentration ≥5×10^6/ml.

Exclusion criteria

Severe male factor infertility, use of donor gametes (oocytes or sperm). Couples were divided into two groups based on male age: Group 1 (<40 years) and Group 2 (≥40 years). Each group was further subdivided according to sperm preparation method: density gradient (DG) or microfluidics (MF). The four subgroups were: A1: age <40, DG (n=56); A2: age <40, MF (n=59); B1: age ≥40, DG (n=33); B2: age ≥40, MF (n=45).

Procedure

Ovarian stimulation and oocyte retrieval

All patients followed a fixed antagonist protocol with a total gonadotropin dose of approximately 3000–3300 IU. Triggering was performed with agonist or dual (agonist + hCG) trigger. Oocyte retrieval was scheduled 34–36 hours later, with an average of 12±2 mature oocytes retrieved.

Sperm preparation methods

Fresh semen samples were collected on the day of oocyte retrieval after 2 days of abstinence. Samples were randomly assigned to one of two preparation methods.

Density gradient

Samples were processed using a discontinuous density gradient (300–400 g for 15–20 minutes), followed by a washing step (200–300 g for 5–10 minutes) to obtain the sperm pellet.

Microfluidics

A centrifugation-free microfluidic chip was used to select highly motile, functionally competent sperm through microchannels, reducing oxidative stress and DNA fragmentation.

ICSI, embryo culture, and frozen embryo transfer: ICSI was performed on all mature MII oocytes. Fertilization was assessed 16–18 hours post-injection. Embryos were cultured to the blastocyst stage and vitrified. Frozen embryo transfers were performed in a subsequent natural or hormone replacement therapy (HRT) cycle.

Endometrial preparation used estradiol valerate (6–10 mg) and, when appropriate, intramuscular progesterone with vaginal micronized progesterone. A single blastocyst was transferred per cycle.

Outcome measures

Primary outcome

Live birth rate (delivery of a neonate at ≥28 weeks gestation). Secondary outcomes: clinical pregnancy rate (presence of gestational sac with cardiac activity on transvaginal ultrasound), biochemical pregnancy rate (positive serum β-hCG >50 IU/l), miscarriage rate (pregnancy loss before 20 weeks), fertilization rate (2PN/oocyte), and blastulation rate (blastocysts/cleaved embryos).

Ethical approval

Ethical approval was obtained from the institutional ethics committee of Nova IVF Fertility prior to the study.

Statistical analysis

Continuous variables (oocytes, MII oocytes, 2PN embryos, blastocysts) were non-normally distributed (Shapiro–Wilk $p<0.05$) and compared using the Mann–Whitney U test. Categorical outcomes were analyzed using Chi-square or Fisher's exact tests, with ORs and 95% confidence intervals (CIs) reported. Multivariate logistic regression evaluated the independent effects of female age (continuous) and subgroup (categorical, reference: A1) on clinical pregnancy. Pairwise subgroup comparisons were performed using Tukey adjustment. A p value <0.05 was considered statistically significant. Analyses were conducted using R version 4.4.1.

RESULTS

Baseline laboratory outcomes and demographic/clinical data of the study participants are detailed in Table 1. No significant differences were observed in oocyte yield, MII oocytes, 2PN embryos, or blastocyst count between DG and MF within the same age group (all $p>0.05$), except for a modest reduction in oocyte yield when comparing younger to older males undergoing MF preparation (A2 vs. B2; $p=0.034$) (Table 2).

Table 1: Laboratory and clinical outcomes by subgroup.

Variables	A1 (n=56)	A2 (n=59)	B1 (n=33)	B2 (n=45)
Oocytes (median, mean±SD)	13, 14.50±8.33	14, 14.88±9.88	10, 11.22±6.73	10, 11.42±7.77
MII (median, mean±SD)	10, 11.93±7.62	11, 12.37±7.84	8, 9.09±5.44	8, 9.78±6.58
2PN (median, mean±SD)	9, 9.30±5.49	9, 9.93±6.20	7, 7.74±4.59	7, 8.24±5.36
Blast (median, mean±SD)	5, 4.84±2.93	4, 5.25±3.47	4, 4.43±2.69	4, 4.04±2.31
CPR (%)	53.23	50.00	44.12	44.44
BPR (%)	12.90	10.29	17.65	7.41
PR (%)	66.13	60.29	61.76	51.85
MR (%)	14.52	5.88	5.88	5.56
LBR (%)	16.13	13.24	17.65	14.81
Fertilization rate (%)	77.99	80.27	82.69	84.32
Blastulation rate (%)	52.02	51.02	54.02	49.06

Table 2: Pairwise comparisons of laboratory and clinical outcomes between subgroups.

Parameters	A1 vs A2	B1 vs B2	A1 vs B1	A2 vs B2
Laboratory (P value)				
Oocytes retrieved	0.95	0.44	0.195	0.034
Mature MII	0.71	0.98	0.22	0.062
2PN	0.75	0.90	0.283	0.142
Blastocyst	0.64	0.92	0.389	0.094
Clinical outcomes				
CPR (P value)	0.35	0.71	0.97	0.09
OR for CPR (95% CI)	1.14 (0.57–2.28)	0.99 (0.41–2.36)	1.43 (0.62–3.38)	1.25 (0.61–2.58)
BPR (P value)	0.64	0.17	0.56	0.75
OR for BPR (95% CI)	1.29 (0.43–3.97)	2.62 (0.67–11.46)	0.69 (0.21–2.34)	1.41 (0.39–5.86)
MR (P value)	0.10	0.99	0.31	0.99
OR for MR (95% CI)	2.64 (0.8–10.58)	1.09 (0.12–7.5)	2.56 (0.6–19.3)	1.05 (0.21–5.92)
LBR (P value)	0.64	0.72	0.84	0.80
OR for LBR (95% CI)	1.26 (0.47–3.44)	1.24 (0.36–4.0)	0.89 (0.29–2.92)	0.88 (0.31–2.55)
Laboratory key parameters				
Fertilization rate (P)	0.29	0.55	0.089	0.082
OR (95% CI)	0.87 (0.67–1.13)	0.89 (0.60–1.32)	0.74 (0.52–1.05)	0.76 (0.55–1.04)
Blastulation rate (P)	0.74	0.16	0.48	0.55
OR (95% CI)	1.04 (0.82–1.32)	1.25 (0.91–1.72)	0.9 (0.67–1.21)	1.08 (0.83–1.4)

Table 3: Multivariate logistic regression for clinical pregnancy by female age and subgroup.

Predictors	OR	95% CI	P value
Female age (continuous)	1.02	0.96–1.09	0.501
Subgroup A2	0.93	0.44–1.97	0.856
Subgroup B1	0.52	0.21–1.31	0.169
Subgroup B2	0.71	0.30–1.68	0.439

Fertilization and blastulation rates were similar across all subgroups. Clinical outcomes did not differ significantly between preparation methods within either age group, nor between age groups using the same preparation method (Table 2). Odds ratios for all clinical outcomes crossed unity.

Multivariate logistic regression (Table 3) showed that female age was not significantly associated with clinical pregnancy (OR 1.02, 95% CI 0.96–1.09, p=0.50). Compared with the reference subgroup A1, no subgroup was a significant predictor of clinical pregnancy (A2: OR 0.93, p=0.86; B1: OR 0.52, p=0.17; B2: OR 0.71, p=0.44). Pairwise comparisons with Tukey adjustment confirmed no significant differences between subgroups (A1 vs. B1: OR 1.91, p=0.515; A1 vs. A2: OR 1.07, p=0.998; B1 vs. B2: OR 0.735, p=0.909).

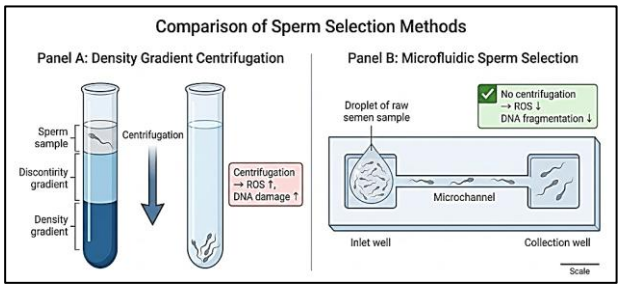

Figure 1: Schematic comparison of density gradient centrifugation versus microfluidic sperm selection. (A) Conventional density gradient method: centrifugation steps may increase reactive oxygen species (ROS) and DNA fragmentation. (B) Microfluidic method: centrifugation-free, gentle selection of motile sperm with reduced oxidative stress.

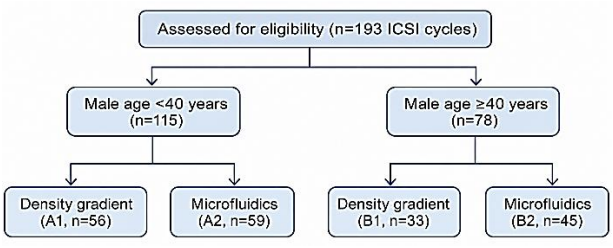

Figure 2: Study flow diagram. Selection of 193 ICSI cycles stratified by male age (<40 years vs. ≥40 years) and sperm preparation method (density gradient centrifugation vs. microfluidics), forming four subgroups (A1, A2, B1, B2).

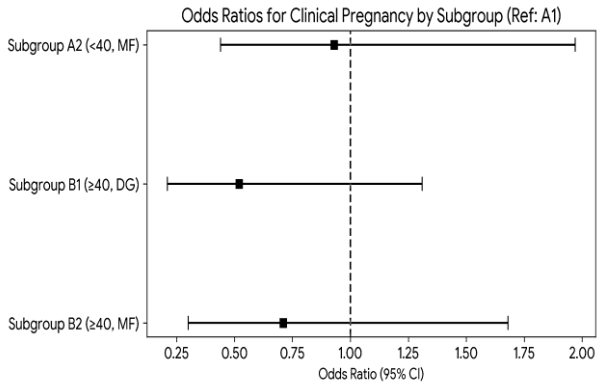

Figure 3: Forest plot of odds ratios for clinical pregnancy by subgroup. Reference subgroup: A1 (male age <40 years, density gradient). Points represent odds ratios (OR) with 95% confidence intervals (error bars). No subgroup shows a statistically significant difference from reference (all p>0.05).

DISCUSSION

In this retrospective cohort of 193 ICSI cycles, neither advanced paternal age (≥40 years) nor the method of sperm preparation (density gradient vs. microfluidics) was independently associated with laboratory or clinical outcomes, including fertilization, blastulation, clinical pregnancy, miscarriage, or live birth. These findings suggest that, under routine ART conditions with controlled maternal age and laboratory protocols, paternal age alone may not be a primary driver of treatment success, however, limitations may be the small sample size and follow up only till clinical pregnancy.

The results are consistent with several contemporary studies that report limited or inconsistent associations between APA and ART outcomes after adjustment for maternal factors.[10,11] Although male aging is associated with measurable biological alterations in sperm cells including increased DNA fragmentation, oxidative stress, and epigenetic changes the clinical translation of these changes into reduced implantation or pregnancy rates remains uncertain.[8,9] The preserved outcomes observed here may reflect the compensatory capacity of ICSI, which can overcome moderate impairments in sperm motility and morphology. Recent investigations in donor-oocyte ICSI cycles, where confounding by oocyte age is minimized, have suggested that APA may negatively influence

blastulation, implantation, and live birth, particularly when conventional sperm processing is used.[12] In such settings, microfluidic selection has been associated with improved outcomes, likely due to enrichment of sperm with lower DNA fragmentation.[7] However, we did not observe a significant benefit of microfluidics over density gradient in either age group. Possible explanations include the absence of severe male factor infertility in our cohort, the lack of SDF data to guide patient selection, and limited statistical power to detect small effect sizes. The isolated reduction in oocyte yield among older males undergoing microfluidic preparation (A2 vs. B2; p=0.034) is unlikely to represent a direct consequence of sperm processing and likely reflects female- or cycle-specific variability.

This finding underscores the complex, multifactorial nature of ART outcomes, in which female reproductive factors continue to exert the predominant influence. Actionable counselling and laboratory strategies for advanced paternal age (≥40 years). Modifiable risk factors (green) and laboratory interventions (blue) to optimise ART outcomes (Figure 4). Actionable points for men aged 40–45 years and older. Modifiable risks before ART include weight, smoking, metabolic health, sleep, and dietary patterns that limit oxidative stress.[1] SDF testing may be considered in selected settings such as repeated blastulation failure or early pregnancy loss, acknowledging mixed evidence.[4] Laboratory practices that minimize reactive oxygen species should be prioritized. Consistent culture and ICSI protocols should be maintained. Paternal age rarely changes the first-line approach but refines risk framing.[10,11]

This study has several limitations its retrospective design introduces potential selection bias; the modest sample size, particularly in older male subgroups, limits statistical power; and the absence of SDF, oxidative stress markers, and epigenetic assessments precludes mechanistic interpretation. Prospective, multi-center studies with larger cohorts are warranted. Incorporation of SDF, OS profiling, and epigenetic markers may help refine individualized sperm selection strategies.[8,9] Long-term follow-up including live birth and neonatal outcomes will enhance IVF treatment planning.

CONCLUSION

In this cohort, sperm preparation method (microfluidics vs. density gradient) and male age (≥40 years) did not independently affect laboratory or clinical outcomes after adjustment for female age. These findings suggest that subgroup classification alone should not guide clinical decision-making regarding sperm preparation in routine ART practice.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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