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Original Research

Association Between Acrosome-Related Sperm Head Abnormalities and Embryo Developmental Competence in First Intracytoplasmic Sperm Injection Cycles: A Retrospective Cohort Study

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ABSTRACT

Introduction: Acrosome-related sperm head abnormalities are frequently encountered during intracytoplasmic sperm injection (ICSI); however, their independent influence on subsequent embryo developmental competence remains uncertain. This study investigated the association between acrosome-related sperm head abnormalities and embryological outcomes in first ICSI cycles after minimizing major female-related confounding factors. **Methods:** This single-center retrospective cohort study analyzed 742 consecutive first ICSI cycles performed between January 2024 and December 2025 in women aged <35 years without severe oocyte dysmorphisms. The cohort comprised 370 cycles exclusively utilizing spermatozoa with acrosome-related head abnormalities (acrosomal area occupying <20% of the sperm head) and 372 control cycles utilizing spermatozoa with normal head morphology. The predefined primary outcome was the blastulation rate per injected mature (metaphase II [MII]) oocyte. Multivariable grouped quasi-binomial logistic regression and negative binomial regression models were fitted to adjust for maternal age, maternal body mass index (BMI), serum anti-Müllerian hormone (AMH) concentration, and controlled ovarian stimulation protocol. **Results:** The primary outcome, blastulation rate, did not differ significantly between the acrosome-related abnormalities group and the control group (median 48% vs. 50%; P = 0.1775). In unadjusted comparisons, cycles utilizing spermatozoa with acrosome-related abnormalities demonstrated significantly lower fertilization rates (84% vs. 88%; P = 0.0085), good-quality cleavage-stage embryo rates (58% vs. 61%; P = 0.0332), and good-quality blastocyst rates (17% vs. 20%; P = 0.0480). After multivariable adjustment, acrosome-related sperm head abnormalities remained independently associated with a reduced fertilization rate (adjusted odds ratio [aOR] 0.86, 95% confidence interval [CI] 0.76–0.98; P = 0.023). Conversely, adjusted associations with the blastulation rate (aOR 0.93, 95% CI 0.83–1.03; P = 0.155), usable blastocyst rate (aOR 0.91, 95% CI 0.82–1.01; P = 0.077), and total number of usable blastocysts per cycle (adjusted incidence rate ratio [aIRR] 0.94, 95% CI 0.86–1.01; P = 0.106) were not statistically significant. Exploratory interaction analyses revealed no significant effect modification by maternal age, ovarian reserve, number of MII oocytes, or ovarian stimulation protocol (all P for interaction > 0.05). **Conclusion:** Acrosome-related sperm head abnormalities were independently associated with a lower fertilization rate in first ICSI cycles after controlling for key female-related factors, whereas multivariable-adjusted associations with blastulation and usable blastocyst rates did not reach statistical significance. While acrosomal defects compromise oocyte fertilization following ICSI, their independent impact on subsequent preimplantation embryo developmental competence appears limited.

Key words: Acrosome, Blastulation rate, Embryo development, Intracytoplasmic sperm injection, Sperm morphology

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) has revolutionized the management of male-factor infertility by bypassing the physiological barriers that impede natural sperm–oocyte interaction[1]. Nevertheless, fertilization and preimplantation embryo development rates remain variable following ICSI, indicating that intrinsic paternal factors continue to influence reproductive outcomes beyond the mechanical delivery of a spermatozoon into the ooplasm. Among the structural components of the sperm head, the acrosome plays an essential role in physiological fertilization and is closely linked to nuclear remodeling and functional maturation during spermiogenesis[2,3]. Although ICSI circumvents the requirement for acrosome-mediated penetration of

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the zona pellucida and cumulus oophorus, morphological defects of the acrosome frequently coexist with underlying ultrastructural or biochemical deficiencies that remain relevant to oocyte activation and subsequent embryonic development.

The prognostic value of conventional sperm morphology for assisted reproductive technology (ART) outcomes remains debated. Whereas routine semen morphology assessments have demonstrated limited ability to predict blastocyst formation following ICSI[4,5], growing evidence suggests that targeted evaluation of specific sperm head defects at high magnification provides superior prognostic insight. Structural abnormalities involving the sperm neck, nucleus, and acrosome have been associated with impaired fertilization, arrested embryo cleavage, and reduced blastulation[6,7]. Acrosome-related abnormalities are of particular clinical and biological interest because aberrant acrosome biogenesis may coincide with defective sperm maturation, oocyte activation deficiency, impaired chromatin condensation, and compromised genomic integrity[8–10]. Specifically, reduced fertilization rates have been documented in men presenting with partial acrosomal hypoplasia, complete absence of the acrosome (globozoospermia), and related acrosomal disorders[11–13]. However, whether acrosome-related sperm head defects independently compromise post-fertilization embryo developmental competence following ICSI remains unclear.

Interpreting the existing literature is further complicated by the substantial contribution of oocyte quality to early embryogenesis. Maternal age, ovarian reserve, ovarian response to stimulation, and cytoplasmic morphology are primary determinants of fertilization, embryonic genome activation, and blastocyst formation, and high-competence oocytes may partially repair or compensate for paternal defects during early cleavage stages[9,14]. Most prior studies have enrolled heterogeneous female cohorts or focused predominantly on fertilization outcomes, making it difficult to isolate the intrinsic contribution of acrosome-related sperm abnormalities from variability in oocyte developmental competence. To minimize female-related confounding, the present study restricted analysis to first ICSI cycles in women aged <35 years with favorable ovarian response and excluded cycles exhibiting severe oocyte dysmorphisms. Accordingly, we aimed to investigate whether acrosome-related sperm head abnormalities are independently associated with embryological outcomes in first ICSI cycles—with the blastulation rate per injected mature oocyte designated as the predefined primary outcome—after minimizing heterogeneity in key female reproductive characteristics.

MATERIALS AND METHODS

Study design and participants

This single-center, retrospective cohort study included consecutive couples undergoing their first ICSI cycle between January 2024 and December 2025 at the Assisted Reproductive Technology Department, An Sinh Hospital (Ho Chi Minh City, Vietnam). In routine clinical practice at our center, ICSI is widely performed as the standard fertilization method during in vitro fertilization (IVF) treatment, including in first cycles without severe oligozoospermia or asthenozoospermia. The clinical decision to perform ICSI was made by the attending physician prior to treatment initiation and was independent of this retrospective investigation. Only cycles utilizing fresh autologous oocytes with a planned indication for extended blastocyst culture were eligible for inclusion. Eligible female partners were younger than 35 years and had no documented medical or gynecological conditions expected to adversely affect ovarian response or oocyte quality.

All included cycles utilized freshly ejaculated autologous semen samples demonstrating normal sperm concentration ($\geq 16 \times 10^6$ spermatozoa/mL) and progressive motility ($\geq$30%) according to the World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Semen (6th edition). Exposure status was defined at the treatment-cycle level rather than the individual spermatozoon level, as real-time sperm selection for ICSI reflects the morphological profile of the entire viable sperm population available at injection. The study group (acrosome-related abnormalities group) comprised cycles in which no morphologically normal spermatozoa could be identified and all spermatozoa selected for injection exhibited severe acrosome-related head abnormalities. The control group consisted of couples meeting identical maternal and seminal eligibility criteria who underwent ICSI using spermatozoa with normal head and acrosomal morphology.

Cycles utilizing cryopreserved sperm, donor sperm, or surgically retrieved sperm—including percutaneous epididymal sperm aspiration (PESA), conventional testicular sperm extraction (TESE), or microdissection TESE (micro-TESE)—were excluded. Additional exclusion criteria were rescue ICSI, predominant sperm morphological defects other than

acrosome-related head abnormalities, severe intracytoplasmic or extracytoplasmic oocyte dysmorphisms known to compromise embryo developmental competence (specifically large or multiple cytoplasmic vacuoles, centrally located cytoplasmic granulation, or smooth endoplasmic reticulum [SER] aggregates), and cycles yielding fewer than six mature (metaphase II [MII]) oocytes for injection. The lower cutoff of six MII oocytes was predefined to reduce variance attributable to suboptimal ovarian response, as prior evidence indicates that cycles retrieving approximately 6–15 mature oocytes exhibit more uniform oocyte developmental competence than low-yield cycles[15]. Each couple contributed only their first treatment cycle to eliminate repeated-measures bias.

The minimum required sample size was calculated using the standard formula for comparing two independent proportions. Based on the historical blastulation rate at our center (52%) and prior evidence linking severe sperm head teratozoospermia to reduced blastocyst formation[16], an expected blastulation rate of 40% was assumed for the acrosome-related abnormalities group. Assuming a two-sided significance level (α) of 0.05 and 80% statistical power ($1 - \beta = 0.80$), a minimum of 270 treatment cycles per group was required.

Controlled ovarian stimulation and ICSI procedures

Controlled ovarian stimulation (COS) was conducted using either a progestin-primed ovarian stimulation (PPOS) protocol or a gonadotropin-releasing hormone (GnRH) antagonist protocol. In routine clinical practice at our center, the attending clinician selected the stimulation regimen based on the planned embryo transfer strategy: the PPOS protocol was preferentially employed when a freeze-all strategy was planned, whereas the GnRH antagonist protocol was selected when fresh embryo transfer was anticipated.

In the PPOS regimen, ovarian stimulation was performed using highly purified menotropin (IVF-M, LG Chem Ltd., Seoul, Republic of Korea) co-administered with oral dydrogesterone (Duphaston, Abbott Biologicals B.V., Olst, the Netherlands; 10 mg twice daily) from the initiation of gonadotropin stimulation until the day of the final oocyte maturation trigger. In the GnRH antagonist regimen, ovarian stimulation was performed using menotropin (IVF-M) with flexible introduction of cetrorelix acetate (Cetrotide, Merck Europe B.V., Amsterdam, the Netherlands). Final oocyte maturation was induced using a dual trigger (triptorelin plus human chorionic gonadotropin) once at least three leading follicles reached a mean diameter of $\geq$17 mm, followed by transvaginal ultrasound-guided oocyte retrieval 36–38 hours later.

During routine clinical practice, real-time sperm selection for ICSI was performed by senior embryologists under an inverted microscope at ×600 magnification. All embryologists performing ICSI were trained under a single standardized laboratory protocol and applied uniform morphological selection criteria throughout the study period. Morphologically normal spermatozoa were preferentially selected whenever present. Acrosomal morphology was evaluated by estimating the proportion of the anterior sperm head occupied by the acrosomal region. A cycle was assigned to the acrosome-related abnormalities group only when no morphologically normal spermatozoa could be identified in the processed sample and every spermatozoon selected for ICSI exhibited an acrosomal area occupying $<$20% of the sperm head (including occasional spermatozoa with complete absence of the acrosome); no cases of complete (100%) globozoospermia were present in the analytic cohort. This $<$20% threshold was predefined because it represents a marked departure from the physiological acrosomal coverage of 40%–70% of the sperm head specified in the WHO Laboratory Manual (6th edition), thereby ensuring clear morphological separation between the exposure and control groups. This conservative institutional criterion was established by consensus among senior embryologists at our center to maximize consistency and minimize subjective misclassification during real-time assessment of unstained, motile spermatozoa. Spermatozoa displaying other major head defects (e.g., macrocephaly, pinhead, or multiple heads) were deemed unsuitable for clinical injection and were excluded from both clinical use and the present analysis.

ICSI was performed on all MII oocytes, and injected oocytes were subsequently cultured in Continuous Single Culture Medium (CSCM; FUJIFILM Irvine Scientific, Santa Ana, CA, USA) through to the blastocyst stage.

Embryo culture and morphological assessment

Fertilization was evaluated 16–18 hours post-ICSI; normal fertilization was defined by the presence of

two distinct pronuclei (2PN) and two polar bodies. Because embryos were cultured in a microdrop group-culture system, individual zygotes could not be tracked longitudinally to the blastocyst stage; consequently, pronuclear morphology (Z-score grading) was not analyzed.

Cleavage-stage embryos were evaluated 67–69 hours post-ICSI in accordance with the Alpha Scientists in Reproductive Medicine and ESHRE Istanbul Consensus criteria. Blastocyst morphology was graded 115–117 hours post-ICSI using the Gardner and Schoolcraft grading system. Good-quality cleavage-stage embryos were defined as grade 1 or grade 2 embryos with stage-appropriate blastomere number and $<$20% fragmentation. Good-quality blastocysts were defined as expanded or hatching blastocysts (expansion grade $\geq$3) with an inner cell mass (ICM) and trophectoderm (TE) graded as AA, AB, BA, or BB (grade 1 or grade 2 institutional equivalent). Usable blastocysts were defined as all blastocysts meeting institutional clinical criteria for either fresh embryo transfer or cryopreservation. In cycles utilizing the PPOS protocol, all usable blastocysts were vitrified. In cycles utilizing the GnRH antagonist protocol, up to two embryos were selected for fresh transfer, and all supernumerary usable blastocysts were vitrified using the Cryotech Vitrification Kit 101 (Cryotech, Tokyo, Japan).

Baseline variables

Baseline clinical and laboratory covariates extracted from electronic medical records included maternal age, paternal age, maternal body mass index (BMI), baseline serum anti-Müllerian hormone (AMH) concentration, COS protocol (PPOS vs. GnRH antagonist), duration of ovarian stimulation (days), total follicle-stimulating hormone (FSH)/menotropin dose administered (IU), total number of retrieved cumulus–oocyte complexes, number of mature (MII) oocytes, ejaculated sperm concentration ($\times 10^6$/mL), and progressive sperm motility (%).

Outcome measures

The predefined primary outcome was the blastulation rate, calculated as the proportion of injected MII oocytes that developed to the blastocyst stage. Predefined secondary outcomes included the normal fertilization rate, cleavage-stage embryo rate, good-quality cleavage-stage embryo rate, good-quality blastocyst rate, usable blastocyst rate, and the absolute number of usable blastocysts generated per treatment cycle.

Specifically, the fertilization rate was defined as the number of 2PN zygotes divided by the total number of injected MII oocytes. The cleavage-stage embryo rate and good-quality cleavage-stage embryo rate were defined as the proportions of injected MII oocytes developing into cleavage-stage embryos and good-quality cleavage-stage embryos on Day 3, respectively. Similarly, the good-quality blastocyst rate and usable blastocyst rate were defined as the proportions of injected MII oocytes developing into good-quality blastocysts and clinically usable blastocysts on Day 5, respectively. All proportional embryological outcomes were computed at the patient (cycle) level using the number of injected MII oocytes as the denominator, irrespective of whether embryos were subsequently transferred fresh or vitrified. In addition, the total number of usable blastocysts obtained per cycle was analyzed as a discrete count outcome.

Statistical analysis

Continuous variables were evaluated for normality using the Shapiro–Wilk test. Normally distributed continuous variables are expressed as mean ± standard deviation (SD) and were compared using the independent-samples Student's t-test; non-normally distributed continuous variables are expressed as median (interquartile range [IQR]) and were compared using the Mann–Whitney U test. Categorical variables are presented as frequency (percentage) and were compared using Pearson's chi-square ($\chi 2$) test or Fisher's exact test, as appropriate.

To account for within-cycle clustering of oocytes and inter-patient variability in MII oocyte yield, multivariable generalized linear models (GLMs) with a quasi-binomial distribution and logit link function were fitted to estimate the independent associations between acrosome-related sperm head abnormalities and the fertilization rate, blastulation rate, and usable blastocyst rate. Outcomes were parameterized as grouped binomial counts (number of successes versus number of failures out of total injected MII oocytes per cycle), with each patient contributing a single independent observation. Multivariable models were adjusted for a priori selected female and treatment covariates: maternal age ($<$30 vs. $\geq$30 years), maternal BMI ($\leq$23.0 vs. $>$23.0 kg/m2), serum AMH concentration ($\leq$4.5 vs. $>$4.5 ng/mL), and COS protocol (GnRH antagonist vs. PPOS). Extra-binomial variation (overdispersion) was quantified using the Pearson dispersion parameter (φ) and accounted for via the quasi-binomial

variance structure. Effect estimates are reported as adjusted odds ratios (aORs) with 95% confidence intervals (CIs). Dichotomous cutoffs for maternal age (<30 vs. ≥30 years), serum AMH concentration (≤4.5 vs. >4.5 ng/mL), and maternal BMI (≤23.0 vs. >23.0 kg/m2) were selected based on established clinical thresholds in reproductive medicine [17,18] and the WHO Western Pacific/Asia-Pacific BMI classification for Asian populations.

To evaluate predictors of the absolute number of usable blastocysts yielded per treatment cycle, a multivariable negative binomial regression model with a log link function was fitted, adjusting for the same set of covariates; results are reported as adjusted incidence rate ratios (aIRRs) with 95% CIs.

Pre-specified exploratory interaction analyses were performed by incorporating multiplicative interaction terms into the quasi-binomial models to test whether the association between acrosome-related sperm head abnormalities and embryological outcomes varied across strata of maternal age (<30 vs. ≥30 years), ovarian reserve (AMH ≤4.5 vs. >4.5 ng/mL), ovarian response (number of MII oocytes, modeled continuously), or COS protocol (PPOS vs. GnRH antagonist).

All statistical analyses were performed using R software (version 4.4.2; R Foundation for Statistical Computing, Vienna, Austria). A two-sided P value < 0.05 was considered statistically significant.

RESULTS

Study cohort and baseline characteristics

A total of 2,453 consecutive first ICSI cycles performed between January 2024 and December 2025 were screened for eligibility. Of these, 349 cycles were excluded because embryos were not cultured to the blastocyst stage, and 1,362 cycles were excluded based on the predefined clinical, seminal, and oocyte exclusion criteria. The final analytic cohort comprised 742 cycles, including 370 cycles in the acrosome-related abnormalities group and 372 cycles in the morphologically normal control group (Figure 1).

Baseline parental demographic characteristics, ovarian reserve markers, gonadotropin stimulation parameters, oocyte yield, and conventional semen parameters were well balanced between the two groups (Table 1). Specifically, no statistically significant differences were observed between the acrosome-related abnormalities group and the control group with respect to maternal age, paternal age, maternal BMI, serum AMH concentration, duration of ovarian stimulation, total gonadotropin dose, number of retrieved oocytes, number of injected MII oocytes, sperm concentration, or progressive sperm motility (all $P > 0.05$). The sole baseline difference between groups was the distribution of COS protocols: a higher proportion of cycles in the acrosome-related abnormalities group utilized the PPOS protocol (57% vs. 34%), whereas the GnRH antagonist protocol was more frequent in the control group (66% vs. 43%; $P < 0.001$) (Table 1).

Unadjusted embryological outcomes

Unadjusted patient-level embryological outcomes are summarized in Table 2. The predefined primary outcome, blastulation rate per injected MII oocyte, did not differ significantly between the acrosome-related abnormalities group and the control group (median 48% [IQR 35%–60%] vs. 50% [IQR 36%–63%]; P = 0.1775).

Among the secondary outcomes, cycles in the acrosome-related abnormalities group exhibited a significantly lower normal fertilization rate (median 84% [IQR 74%–92%] vs. 88% [IQR 78%–93%]; P = 0.0085), good-quality cleavage-stage embryo rate (58% [IQR 46%–70%] vs. 61% [IQR 48%–75%]; P = 0.0332), and good-quality blastocyst rate (17% [IQR 8%–29%] vs. 20% [IQR 11%–31%]; P = 0.0480) compared with the control group. Conversely, no statistically significant between-group differences were detected in the overall cleavage-stage embryo rate (72% [IQR 62%–82%] vs. 74% [IQR 63%–85%]; P = 0.1471) or the usable blastocyst rate (38% [IQR 25%–50%] vs. 38% [IQR 27%–50%]; P = 0.1283) (Table 2).

Multivariable quasi-binomial regression analyses

Multivariable grouped quasi-binomial logistic regression models were fitted to evaluate the independent associations between acrosome-related sperm head abnormalities and proportional embryological outcomes after adjusting for maternal age, maternal BMI, serum AMH concentration, and COS protocol. For the primary outcome, acrosome-related sperm head abnormalities were not significantly associated with the blastulation rate after multivariable adjustment (aOR 0.93, 95% CI 0.83–1.03; P = 0.155) (Table 3a).

For the secondary proportional outcomes, acrosome-related sperm head abnormalities remained independently associated with a 14% lower odds of normal oocyte fertilization per

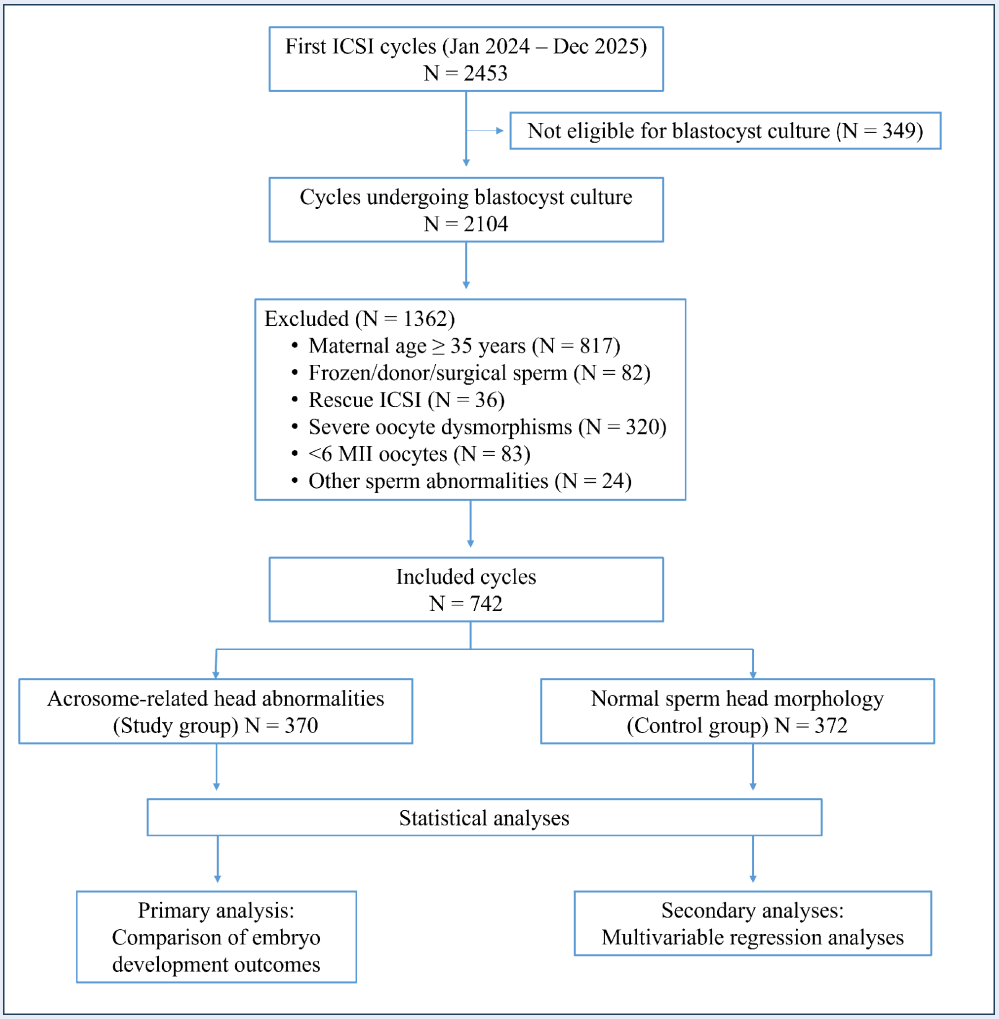


Figure 1: Patient screening, eligibility assessment, and cohort allocation flowchart for first intracytoplasmic sperm injection (ICSI) cycles performed at An Sinh Hospital between January 2024 and December 2025. A total of 2,453 consecutive first ICSI cycles in women aged <35 years were screened. Following the exclusion of cycles without blastocyst culture (n = 349) and cycles meeting predefined clinical, seminal, or oocyte exclusion criteria (n = 1,362; including frozen, donor, or surgically retrieved sperm [PESA, TESE, or micro-TESE], rescue ICSI, non-acrosomal sperm head defects, severe oocyte dysmorphisms, or <6 mature metaphase II [MII] oocytes retrieved), 742 eligible cycles were included in the final analysis. Eligible cycles were stratified into the acrosome-related sperm head abnormalities group (acrosomal area occupying <20% of the sperm head across all injected spermatozoa; n = 370) and the morphologically normal control group (normal sperm head and acrosomal morphology; n = 372). Abbreviations: ICSI, intracytoplasmic sperm injection; micro-TESE, microdissection testicular sperm extraction; MII, metaphase II; PESA, percutaneous epididymal sperm aspiration; TESE, testicular sperm extraction.

injected MII oocyte (aOR 0.86, 95% CI 0.76–0.98; P = 0.023). By contrast, the multivariable-adjusted association between acrosome-related abnormalities and the usable blastocyst rate was attenuated and did not reach statistical significance (aOR 0.91, 95% CI 0.82–1.01; P = 0.077) (Table 3b). None of the adjusted female covariates (maternal age, BMI, AMH concentration, or COS protocol) showed a statistically significant association with fertilization rate, blastulation rate, or usable blastocyst rate in the grouped quasi-binomial models. The estimated Pearson dispersion parameters were φ = 1.68 for the fertilization rate, φ = 2.10 for the blastulation rate, and φ = 1.85 for the usable blastocyst rate, confirming moderate overdispersion that was

Table 1: Baseline demographic, ovarian stimulation, oocyte, and conventional semen characteristics of couples undergoing first ICSI cycles stratified by sperm acrosomal morphology (N = 742).

Characteristic	Control Group (Normal Sperm Head Morphology, n = 372)	Study Group (Acrosome-Related Sperm Head Abnormalities, n = 370)	P value
Continuous variables, median (IQR)			
Maternal age (years)	31 (28–33)	31 (28–34)	0.09
Paternal age (years)	33 (30–37)	34 (31–37)	0.20
Maternal BMI (kg/m²)	21.84 (20.00–23.63)	22.00 (20.05–23.83)	0.80
Serum AMH concentration (ng/mL)	4.08 (2.64–6.07)	3.94 (2.43–6.09)	0.50
Ovarian stimulation duration (days)	8 (8–9)	8 (8–9)	0.50
Total gonadotropin (FSH) dose (IU)	2,400 (2,400–2,700)	2,400 (2,400–2,700)	0.80
Retrieved oocytes per cycle (n)	19 (13–25)	18 (13–25)	0.60
Mature (MII) oocytes per cycle (n)	15 (11–20)	15 (11–20)	0.80
Sperm concentration (×10⁶/mL)	35 (25–45)	35 (25–45)	0.90
Progressive sperm motility (%)	50 (39–63)	50 (39–63)	0.90
Controlled ovarian stimulation protocol, n (%)			<0.001
PPOS protocol	125 (34%)	212 (57%)	
GnRH antagonist protocol	247 (66%)	158 (43%)	

Legend: Data are presented as median (interquartile range [IQR]) for non-normally distributed continuous variables and as frequency (percentage) for categorical variables. Between-group comparisons were conducted using the two-sided Mann–Whitney U test for continuous variables and Pearson's chi-square (χ^2) test for categorical variables. Statistically significant P values (<0.05) are shown. Abbreviations: AMH, anti-Müllerian hormone; BMI, body mass index; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; ICSI, intracytoplasmic sperm injection; IQR, interquartile range; MII, metaphase II; PPOS, progestin-primed ovarian stimulation.

Table 2: Unadjusted comparison of patient-level fertilization and preimplantation embryo development rates per injected metaphase II oocyte between the acrosome-related abnormalities group and the morphologically normal control group (N = 742).

Embryological Outcome	Control Group (Normal Sperm Head Morphology, n = 372)	Study Group (Acrosome-Related Sperm Head Abnormalities, n = 370)	P value
Primary outcome			
Blastulation rate (%)	50 (36–63)	48 (35–60)	0.1775
Secondary outcomes			
Normal fertilization (2PN) rate (%)	88 (78–93)	84 (74–92)	0.0085
Cleavage-stage embryo rate (%)	74 (63–85)	72 (62–82)	0.1471
Good-quality cleavage-stage embryo rate (%)	61 (48–75)	58 (46–70)	0.0332
Good-quality blastocyst rate (%)	20 (11–31)	17 (8–29)	0.0480
Usable blastocyst rate (%)	38 (27–50)	38 (25–50)	0.1283

Data are expressed as median percentage (interquartile range [IQR]). All proportional embryological outcomes were calculated at the individual cycle level using the total number of injected mature (metaphase II [MII]) oocytes as the denominator. Between-group comparisons were performed using the two-sided Mann–Whitney U test. Good-quality embryos were defined as grade 1 or grade 2 on Day 3 (Alpha/ESHRE consensus) or Day 5 (Gardner grading system), and usable blastocysts included all blastocysts suitable for fresh transfer or vitrification. Abbreviations: 2PN, two pronuclei; ICSI, intracytoplasmic sperm injection; IQR, interquartile range; MII, metaphase II.

Table 3a: Multivariable grouped quasi-binomial logistic regression analysis of factors associated with the primary outcome (blastulation rate per injected metaphase II oocyte) in first ICSI cycles (N = 742).

Variable / Category	Blastulation Rate aOR (95% CI)	P value
Sperm acrosome morphology		
Normal (≥40%–70% head coverage)	1.00 (Reference)	—
Abnormal (<20% head coverage)	0.93 (0.83–1.03)	0.155
Maternal age (years)		
<30	1.00 (Reference)	—
≥30	0.93 (0.84–1.04)	0.207
Maternal BMI (kg/m²)		
≤23.0	1.00 (Reference)	—
>23.0	0.92 (0.82–1.03)	0.130
Serum AMH concentration (ng/mL)		
≤4.5	1.00 (Reference)	—
>4.5	1.01 (0.91–1.12)	0.852
Controlled ovarian stimulation protocol		
GnRH antagonist	1.00 (Reference)	—
PPOS	1.06 (0.95–1.18)	0.312

Effect estimates were derived from a multivariable generalized linear model (GLM) with a quasi-binomial distribution and logit link function (Pearson dispersion parameter φ = 2.10), modeling the number of blastocysts formed out of injected mature (metaphase II [MII]) oocytes per cycle while simultaneously adjusting for all listed covariates. Abbreviations: AMH, anti-Müllerian hormone; aOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; GLM, generalized linear model; GnRH, gonadotropin-releasing hormone; ICSI, intracytoplasmic sperm injection; MII, metaphase II; PPOS, progestin-primed ovarian stimulation.

appropriately accounted for by the quasi-binomial variance specification.

Multivariable negative binomial regression analysis of usable blastocyst count

Because proportional rates per MII oocyte do not directly capture the cumulative number of embryos available for clinical transfer or cryopreservation per cycle, a multivariable negative binomial regression model was fitted to evaluate the total count of usable blastocysts obtained per cycle (Table 4).

After adjustment for maternal age, maternal BMI, serum AMH concentration, and COS protocol, acrosome-related sperm head abnormalities were not independently associated with the number of usable blastocysts yielded per cycle (aIRR 0.94, 95% CI 0.86–1.01; P = 0.106). Conversely, maternal age ≥30 years (aIRR 0.87, 95% CI 0.80–0.94; P < 0.001) and baseline serum AMH concentration >4.5 ng/mL (aIRR 0.92, 95% CI 0.85–1.00; P = 0.038) were independently associated with a lower count of usable blastocysts per cycle. Maternal BMI and COS protocol were not significantly associated with usable blastocyst count (Table 4).

Subgroup interaction analyses

Exploratory interaction models demonstrated no statistically significant effect modification between acrosome-related sperm head abnormalities and maternal age (<30 vs. ≥30 years), baseline serum AMH concentration (≤4.5 vs. >4.5 ng/mL), ovarian response (number of MII oocytes), or COS protocol (PPOS vs. GnRH antagonist) for the fertilization rate, blastulation rate, or usable blastocyst rate (all P for interaction > 0.05; Table 5).

DISCUSSION

Principal findings

The principal finding of this retrospective cohort study is that severe acrosome-related sperm head abnormalities (<20% acrosomal coverage) were independently associated with a lower normal fertilization rate in first ICSI cycles after controlling for key female-related confounding variables. Although cycles utilizing spermatozoa with acrosome-related abnormalities also displayed lower unadjusted rates of good-quality cleavage-stage embryos and good-quality blastocysts, neither the overall cleavage-stage embryo rate nor the primary outcome—blastulation rate—differed significantly between groups. Furthermore, after multivariable adjustment, associations between acrosome-related abnormalities and the blastulation rate, usable blastocyst rate, and total number of usable blastocysts per cycle were not statistically significant. Collectively, these results indicate that while acrosome-related sperm head defects independently impair fertilization following ICSI, fertilized zygotes that successfully progress past pronuclear formation retain largely preserved competence to reach the blastocyst stage.

In the multivariable count model, maternal age ≥30 years was independently associated with a 13% reduction in the number of usable blastocysts per cycle (aIRR 0.87, 95% CI 0.80–0.94; P < 0.001), consistent with age-related declines in oocyte yield and

Table 3b: Multivariable grouped quasi-binomial logistic regression analyses of factors associated with secondary proportional outcomes (normal fertilization rate and usable blastocyst rate per injected metaphase II oocyte) in first ICSI cycles (N = 742).

Variable / Category	Normal Fertilization Rate aOR (95% CI)	P value	Usable Blastocyst Rate aOR (95% CI)	P value
Sperm acrosome morphology				
Normal	1.00 (Reference)	—	1.00 (Reference)	—
Abnormal (<20% head coverage)	0.86 (0.76–0.98)	0.023	0.91 (0.82–1.01)	0.077
Maternal age (years)				
<30	1.00 (Reference)	—	1.00 (Reference)	—
≥30	0.92 (0.81–1.05)	0.208	0.97 (0.87–1.07)	0.514
Maternal BMI (kg/m²)				
≤23.0	1.00 (Reference)	—	1.00 (Reference)	—
>23.0	1.02 (0.89–1.16)	0.817	1.01 (0.91–1.12)	0.842
Serum AMH concentration (ng/mL)				
≤4.5	1.00 (Reference)	—	1.00 (Reference)	—
>4.5	1.10 (0.97–1.26)	0.134	0.96 (0.86–1.06)	0.421
Controlled ovarian stimulation protocol				
GnRH antagonist	1.00 (Reference)	—	1.00 (Reference)	—
PPOS	1.08 (0.95–1.22)	0.270	1.03 (0.93–1.14)	0.586

Effect estimates were derived from multivariable generalized linear models (GLMs) with a quasi-binomial distribution and logit link function (Pearson dispersion parameters: φ = 1.68 for fertilization rate and φ = 1.85 for usable blastocyst rate), modeling grouped binomial counts per injected mature (metaphase II [MII]) oocyte while simultaneously adjusting for all listed covariates. Abbreviations: AMH, anti-Müllerian hormone; aOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; GLM, generalized linear model; GnRH, gonadotropin-releasing hormone; ICSI, intracytoplasmic sperm injection; MII, metaphase II; PPOS, progestin-primed ovarian stimulation.

quality even among women younger than 35 years. Notably, a baseline serum AMH concentration >4.5 ng/mL was also associated with a marginally lower number of usable blastocysts (aIRR 0.92, 95% CI 0.85–1.00; P = 0.038). This finding may reflect residual confounding by subclinical polycystic ovary morphology or reduced per-oocyte developmental competence in hyper-responders rather than a direct deleterious effect of elevated ovarian reserve, and should be interpreted cautiously given the retrospective design and the absence of MII oocyte count as an offset in the negative binomial model.

Blastulation versus morphological embryo quality following ICSI

An intriguing observation of our study is that overall blastulation rates were comparable between groups, whereas unadjusted rates of good-quality cleavage-stage embryos and good-quality blastocysts were modestly lower in cycles affected by acrosome-related sperm abnormalities. This divergence highlights that blastocyst cavitation and morphological grading capture distinct dimensions of preimplantation development: the blastulation rate reflects the capacity of an fertilized oocyte to undergo compaction, cavitation, and blastocoel expansion, whereas good-quality blastocyst metrics additionally require cohesive inner cell mass organization and uniform trophectoderm cellularity.

Nevertheless, these unadjusted differences in morphological embryo quality should be interpreted with caution. The multivariable-adjusted association between acrosome-related sperm abnormalities and the clinically actionable endpoint—the usable blastocyst rate—did not reach statistical significance (P = 0.077), and secondary quality comparisons were unadjusted for multiple testing. Consequently, our data do not support a definitive independent deleterious effect of acrosome-related sperm abnormalities on post-fertilization embryo developmental competence.

Table 4: Multivariable negative binomial regression analysis of clinical and seminal factors associated with the absolute number of usable blastocysts obtained per treatment cycle in first ICSI cycles (N = 742).

Variable / Category	Usable Blastocyst Count per Cycle aIRR (95% CI)	P value
Sperm acrosome morphology		
Normal	1.00 (Reference)	—
Abnormal (<20% head coverage)	0.94 (0.86–1.01)	0.106
Maternal age (years)		
<30	1.00 (Reference)	—
≥30	0.87 (0.80–0.94)	<0.001
Maternal BMI (kg/m²)		
≤23.0	1.00 (Reference)	—
>23.0	1.03 (0.94–1.12)	0.542
Serum AMH concentration (ng/mL)		
≤4.5	1.00 (Reference)	—
>4.5	0.92 (0.85–1.00)	0.038
Controlled ovarian stimulation protocol		
GnRH antagonist	1.00 (Reference)	—
PPOS	0.97 (0.90–1.06)	0.520

Effect estimates were obtained from a multivariable negative binomial regression model with a log link function evaluating the discrete count of clinically usable blastocysts (suitable for fresh transfer or vitrification) generated per cycle, simultaneously adjusted for maternal age, maternal BMI, serum AMH concentration, and stimulation protocol. Abbreviations: aIRR, adjusted incidence rate ratio; AMH, anti-Müllerian hormone; BMI, body mass index; CI, confidence interval; GnRH, gonadotropin-releasing hormone; ICSI, intracytoplasmic sperm injection; PPOS, progestin-primed ovarian stimulation.

Table 5: Exploratory subgroup interaction analyses evaluating effect modification by maternal age, oocyte yield, ovarian reserve, and ovarian stimulation protocol on the association between acrosome-related sperm head abnormalities and embryological outcomes (N = 742).

Clinical Effect Modifier	P for Interaction (Normal Fertilization Rate)	P for Interaction (Blastulation Rate)	P for Interaction (Usable Blastocyst Rate)
Maternal age (<30 vs. ≥30 years)	0.566	0.248	0.575
Ovarian response (number of MII oocytes, continuous)	0.393	0.134	0.294
Serum AMH concentration (≤4.5 vs. >4.5 ng/mL)	0.420	0.537	0.951
Controlled ovarian stimulation protocol (PPOS vs. GnRH antagonist)	0.623	0.680	0.487

P values for interaction were derived from multivariable grouped quasi-binomial generalized linear models containing main effects (sperm acrosomal morphology, maternal age, maternal BMI, serum AMH concentration, and stimulation protocol) plus a multiplicative two-way interaction term between sperm acrosomal morphology (abnormal vs. normal) and each specified clinical modifier. All P for interaction values exceeded 0.05, indicating no statistically significant effect modification across clinical strata. Abbreviations: AMH, anti-Müllerian hormone; BMI, body mass index; GnRH, gonadotropin-releasing hormone; MII, metaphase II; PPOS, progestin-primed ovarian stimulation.

Comparison with previous studies

The independent reduction in fertilization rate observed in our cohort aligns closely with the established physiological role of the acrosome and perinuclear theca in sperm-borne oocyte activation. Prior clinical investigations have consistently documented compromised fertilization or total fertilization failure in men with partial acrosomal hypoplasia, small acrosomes, or globozoospermia, even when mechanical sperm delivery via ICSI is employed [11–13,19]. By restricting our study population to young women (<35 years) with normal ovar-

ian response and morphologically normal oocytes, our study extends prior observations by confirming that this fertilization deficit persists independently of female-related factors.

Interestingly, the pattern observed in our unadjusted analyses—preserved overall blastulation accompanied by a modest reduction in high-grade blastocysts—parallels findings from recent studies of elevated sperm DNA fragmentation, wherein overall blastocyst formation remained largely unaffected while embryo morphokinetics and the yield of top-quality blastocysts were compromised [20, 21]. However, because our multivariable analyses showed no statistically significant reduction in either overall blastulation or usable blastocyst yield, any downstream paternal effect of acrosomal abnormalities after successful fertilization appears modest when high-quality young oocytes are utilized.

Biological plausibility and putative mechanisms

From a developmental biology perspective, subtle impairments in cleavage and blastocyst morphology have been attributed to the 'late paternal effect,' whereby paternal genomic or epigenetic lesions manifest after embryonic genome activation at the 4- to 8-cell transition[20–22]. Furthermore, high-competence oocytes from young women possess robust maternal mRNA transcripts and DNA repair machinery capable of correcting moderate paternal chromatin lesions during the first cleavage division[14,21,22], which may explain why fertilized oocytes in our <35-year female cohort achieved near-normal blastulation rates.

With respect to fertilization impairment, acrosome biogenesis occurs concurrently with nuclear elongation and histone-to-protamine replacement during spermiogenesis. Structural defects in acrosomal vesicle biogenesis are closely linked to perturbations in the perinuclear theca, resulting in reduced expression or abnormal localization of phospholipase C zeta (PLCζ)—the primary physiological sperm factor responsible for inducing intracellular calcium (Ca2+) oscillations and metaphase-to-anaphase transition in the MII oocyte[8,10,13,19]. Additionally, aberrant acrosomal morphogenesis has been associated with incomplete protamination, loosely packaged chromatin, and elevated sperm DNA fragmentation[10,13,19]. Because our retrospective study did not directly assay PLCζ expression, chromatin protamination, or sperm DNA fragmentation index (DFI), these mechanistic pathways remain hypothetical and warrant verification in prospective translational studies.

Clinical implications

Our findings have practical implications for patient counseling and laboratory management in ART. When embryologists encounter ejaculates in which all available spermatozoa exhibit severe acrosomal reduction (<20% of the head area) during routine ICSI, clinicians can counsel couples that although a modest reduction in the normal fertilization rate is anticipated, the likelihood that fertilized oocytes will progress into clinically usable blastocysts remains reassuringly favorable, particularly in women aged <35 years with good ovarian reserve.

Moreover, the selective impairment in fertilization provides a clear biological rationale for future randomized controlled trials investigating whether targeted adjuvants—such as assisted oocyte activation (AOA) with calcium ionophores or advanced microfluidic/high-magnification sperm selection (IMSI)—can normalize fertilization rates and optimize cumulative blastocyst yield in patients with severe acrosomal abnormalities.

Strengths and limitations

A major strength of the present study is its rigorous eligibility design, which was specifically structured to minimize female-related confounding. By restricting the cohort to first ICSI cycles in women younger than 35 years with at least six MII oocytes and excluding cycles with severe cytoplasmic or extracytoplasmic oocyte dysmorphisms, we substantially reduced variability in maternal oocyte competence. Furthermore, we employed grouped quasi-binomial GLMs to account for within-patient clustering of oocytes and extra-binomial overdispersion, alongside negative binomial modeling for embryo counts and formal interaction testing.

Although the proportion of PPOS versus GnRH antagonist cycles differed at baseline between the two groups, COS protocol was adjusted for in all multivariable models and showed neither an independent association with embryological outcomes nor any statistically significant interaction with acrosomal morphology (all P for interaction > 0.48), ruling out meaningful confounding or effect modification by stimulation regimen.

Several limitations must also be considered. First, inherent to all observational retrospective studies, residual confounding by unmeasured paternal or lifestyle factors cannot be entirely excluded, and analyses of multiple secondary outcomes were not adjusted for multiplicity and should therefore be viewed as exploratory. Second, acrosomal morphology was evaluated visually at ×600 magnification

during routine clinical ICSI rather than via transmission electron microscopy, acrosomal staining, or computer-assisted morphometric analysis. Although a stringent, conservative institutional cut-off (<20% acrosomal coverage across all injected sperm) was applied to maximize separation from normal controls, formal inter- and intra-observer kappa reliability metrics were not recorded, leaving potential for minor observer-dependent variability. Third, classifying acrosome-related defects as a dichotomous exposure precluded dose–response modeling across varying degrees of acrosomal hypoplasia. Fourth, molecular assays of sperm DNA fragmentation, chromatin packaging, and PLCζ localization were not performed. Finally, clinical transfer outcomes—including biochemical pregnancy, clinical pregnancy, miscarriage, and live birth rates—were beyond the scope of this embryological analysis. Prospective studies integrating quantitative morphometrics, functional sperm biomarkers, and cumulative live birth outcomes are warranted.

CONCLUSION

In conclusion, severe acrosome-related sperm head abnormalities were independently associated with a lower normal fertilization rate in first ICSI cycles after controlling for key female-related factors. Although unadjusted comparisons showed slightly lower rates of good-quality cleavage-stage embryos and good-quality blastocysts, multivariable-adjusted associations with the primary outcome (blastulation rate), usable blastocyst rate, and usable blastocyst count per cycle were not statistically significant. These findings indicate that while acrosome-related sperm head defects impair oocyte fertilization following ICSI, their independent impact on subsequent blastocyst developmental competence in young women with favorable oocyte quality is limited. Prospective studies incorporating quantitative acrosomal morphometry, functional sperm biomarkers, and live birth outcomes are warranted.

DECLARATIONS

Abbreviations

2PN: Two pronuclei; aIRR: Adjusted incidence rate ratio; AMH: Anti-Müllerian hormone; AOA: Assisted oocyte activation; aOR: Adjusted odds ratio; ART: Assisted reproductive technology; BMI: Body mass index; CI: Confidence interval; COS: Controlled ovarian stimulation; CSCM: Continuous Single Culture Medium; DFI: DNA fragmentation index; ESHRE: European Society of Human Reproduction and Embryology; FSH: Follicle-stimulating hormone; GLM: Generalized linear model; GnRH: Gonadotropin-releasing hormone; ICM: Inner cell mass; ICSI: Intracytoplasmic sperm injection; IMSI: Intracytoplasmic morphologically selected sperm injection; IQR: Interquartile range; IVF: In vitro fertilization; micro-TESE: Microdissection testicular sperm extraction; MII: Metaphase II; PESA: Percutaneous epididymal sperm aspiration; PLCζ (PLCZ1): Phospholipase C zeta; PPOS: Progestin-primed ovarian stimulation; SD: Standard deviation; SER: Smooth endoplasmic reticulum; TE: Trophectoderm; TESE: Testicular sperm extraction; WHO: World Health Organization.

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Author's contributions

Truc Nhu Thanh Vo: Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. Trang Thao Vu, Khanh Thi Mai Nguyen, and Trang Thi Thuy Nguyen: Investigation, Data curation. Huong Thi Thu Hoang: Formal analysis. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author upon reasonable request and subject to institutional approval by An Sinh Hospital.

Ethics approval and consent to participate

This study was reviewed and approved by the Institutional Review Board of An Sinh Hospital (Approval No. 755-26/AS-GCN; dated 10 June 2026). Retrospective data extraction and anonymized analysis were conducted in accordance with institutional guidelines and the Declaration of Helsinki. The requirement for individual written informed consent was waived by the Institutional Review Board owing to the retrospective nature of the study.

Consent for publication

None (Not applicable; the manuscript contains no individual person's identifying data or images).

Declaration of generative AI and AI-assisted technologies in the writing process

None.

Competing interests

None. The authors declare that they have no competing interests.

REFERENCES

1. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 1992;340(8810):17–18. PMID: 1351601. Available from: https://doi.org/10.1016/0140-6736(92)92425-F.
2. Yanagimachi R. Fertility of mammalian spermatozoa: its development and relativity. Zygote. 1994;2(4):371–372. PMID: 8665172. Available from: https://doi.org/10.1017/S0967199400002240.
3. Khawar MB, Gao H, Li W. Mechanism of acrosome biogenesis in mammals. Frontiers in Cell and Developmental Biology. 2019;7:195. PMC6759746. PMID: 31620437. Available from: https://doi.org/10.3389/fcell.2019.00195.
4. Miller JE, Smith TT. The effect of intracytoplasmic sperm injection and semen parameters on blastocyst development in vitro. Human Reproduction. 2001;16(5):918–924. PMID: 11331638. Available from: https://doi.org/10.1093/humrep/16.5.918.
5. French DB, Sabanegh ESJ, Goldfarb J, Desai N. Does severe teratozoospermia affect blastocyst formation, live birth rate, and other clinical outcome parameters in ICSI cycles? Fertility and Sterility. 2010;93(4):1097–1103. PMID: 19200957. Available from: https://doi.org/10.1016/j.fertnstert.2008.11.022.
6. De Vos A, Van De Velde H, Joris H, Verheyen G, Devroey P, Van Steirteghem A. Influence of individual sperm morphology on fertilization, embryo morphology, and pregnancy outcome of intracytoplasmic sperm injection. Fertility and Sterility. 2003;79(1):42–48. PMID: 12524062. Available from: https://doi.org/10.1016/S0015-0282(02)04571-5.
7. Setti AS, Figueira RC, Braga DP, Aoki T, Iaconelli AJ, Borges EJ. Sperm morphological abnormalities visualised at high magnification predict embryonic development, from fertilisation to the blastocyst stage, in couples undergoing ICSI. Journal of Assisted Reproduction and Genetics. 2014;31(11):1533–1539. PMC4389935. PMID: 25179619. Available from: https://doi.org/10.1007/s10815-014-0326-9.
8. Jones C, Meng X, Coward K. Sperm factors and egg activation: Phospholipase C zeta (PLCZ1) and the clinical diagnosis of oocyte activation deficiency. Reproduction. 2022;164(1):F53–F66. PMID: 35536026. Available from: https://doi.org/10.1530/REP-21-0458.
9. Yeste M, Jones C, Amdani SN, Patel S, Coward K. Oocyte activation deficiency: a role for an oocyte contribution? Human Reproduction Update. 2016;22(1):23–47. PMID: 26346057. Available from: https://doi.org/10.1093/humupd/dmv040.
10. Yoon SY, Jellerette T, Salicioni AM, Lee HC, Yoo MS, Coward K, et al. Human sperm devoid of PLC, zeta 1 fail to induce Ca(2+) release and are unable to initiate the first step of embryo development. Journal of Clinical Investigation. 2008;118(11):3671–3681. PMC2569000. PMID: 18924610. Available from: https://doi.org/10.1172/JCI36942.
11. Dam AH, Feenstra I, Westphal JR, Ramos L, van Golde RJ, Kremer JA. Globozoospermia revisited. Human Reproduction Update. 2007;13(1):63–75. PMID: 17008355. Available from: https://doi.org/10.1093/humupd/dml047.
12. Li C, Sun Y, Zhang X, Wang J, Liu H, Chen Z, et al. The correlation between sperm percentage with a small acrosome and unexplained in vitro fertilization failure. BMC Pregnancy and Childbirth. 2024;24(1):58. PMC10782732. PMID: 38212708. Available from: https://doi.org/10.1186/s12884-024-06246-7.
13. Zahiri Z, Ghasemian F. Is it necessary to focus on morphologically normal acrosome of sperm during intracytoplasmic sperm injection? Indian Journal of Medical Research. 2019;150(5):477–485. PMC6977368. PMID: 31939391. Available from: https://doi.org/10.4103/ijmr.IJMR_1760_17.
14. Bashiri Z, Amiri I, Ghorbani M, Hafezi M, Asgari H, Koruji M. Male factors: the role of sperm in preimplantation embryo quality. Reproductive Sciences. 2021;28(7):1788–1811. PMID: 33140229. Available from: https://doi.org/10.1007/s43032-020-00334-z.
15. Ji J, Liu Y, Tong XH, Luo L, Ma J, Chen Z. The optimum number of oocytes in IVF treatment: an analysis of 2455 cycles in China. Human Reproduction. 2013;28(10):2728–2734. PMID: 23887075. Available from: https://doi.org/10.1093/humrep/det303.
16. Grow DR, Oehninger S, Seltman HJ, Toner JP, Swanson RJ, Kruger TF, et al. Sperm morphology as diagnosed by strict criteria: probing the impact of teratozoospermia on fertilization rate and pregnancy outcome in a large in vitro fertilization population. Fertility and Sterility. 1994;62(3):559–567. PMID: 8062952. Available from: https://doi.org/10.1016/S0015-0282(16)56946-5.
17. Heidary Z, Zare M, Ghaemi M, Rashidi BH, Hantoushzadeh S. The association of AMH level with the number and quality of oocytes in women undergoing IVF/ICSI: a single-center study. Journal of Reproduction and Infertility. 2024;25(1):38–45. PMC11066402. PMID: 38706675. Available from: https://doi.org/10.18502/jri.v25i1.15201.
18. Seifer DB, Baker VL, Leader B. Age-specific serum anti-Müllerian hormone values for 17,120 women presenting to fertility centers within the United States. Fertility and Sterility. 2011;95(2):747–750. PMID: 21074758. Available from: https://doi.org/10.1016/j.fertnstert.2010.10.011.
19. Nasr-Esfahani MH, Razavi S, Tavalaee M. Failed fertilization after ICSI and spermiogenic defects. Fertility and Sterility. 2008;89(4):892–898. PMID: 17603051. Available from: https://doi.org/10.1016/j.fertnstert.2007.04.012.
20. Ye D, Ye Z, Zhang H. Effects of sperm DNA fragmentation on embryo morphokinetic parameters and laboratory outcomes in women of different ages during intracytoplasmic sperm treatment cycles. Archives of Gynecology and Obstetrics. 2025;312(1):191–196. PMC12177009. PMID: 39955692. Available from: https://doi.org/10.1007/s00404-025-07949.
21. Karabulut S, et al. Impact of maternal age on the repairing capacity of oocytes on paternal DNA damage. Reproductive Sciences. 2025;32(7):2397–2403. PMID: 40504287. Available from: https://doi.org/10.1007/s43032-025-01911-w.
22. Ménézo Y, Dale B, Cohen M. DNA damage and repair in human oocytes and embryos: a review. Zygote. 2010;18(4):357–365. PMID: 20663262. Available from: https://doi.org/10.1017/S0967199410000286.