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ORIGINAL RESEARCH

Impact of Clinical and Treatment Factors on Clinical Pregnancy and Live Birth in IVF/ICSI-ET: A Retrospective Cohort Study

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Objective: This study aimed to evaluate the impact of clinical, hormonal, and embryologic factors on clinical pregnancy and live birth outcomes in in vitro fertilization/intracytoplasmic sperm injection and embryo transfer (IVF/ICSI-ET).

Methods: This retrospective cohort analysis included Clinical, endocrine, and embryological data in 178 IVF/ICSI-ET cycles performed at a single reproductive medicine center from January 2020 to December 2024. Clinical pregnancy and live birth served as the primary outcomes. Statistical analyses included univariate and multivariate logistic regression and ROC curve analysis assessing the discriminative abilityof male age.

Results: Among the 139 IVF/ICSI-ET cycles meeting inclusion criteria, the clinical pregnancy rate was 51.8%, with a live birth rate of 38.1%. The pregnancy group exhibited significantly younger male age, lower basal AMH, shorter infertility duration, and more double-embryo transfers (all $p < 0.05$). Multivariate analysis identified male age as the sole independently associated factor of clinical pregnancy (OR = 0.85). ROC analysis indicated moderate discriminative abilityat a cut-off of 31.5 years (AUC = 0.620). Among pregnant patients, live birth was associated with younger female and male age, lower gonadotropin doses, and double-embryo transfer in univariate analysis ($p < 0.05$), but no independently associated factors were confirmed on multivariate analysis ($p > 0.05$).

Conclusion: Male age appears to play a significant role in clinical pregnancy outcomes in IVF/ICSI-ET, highlighting the importance of incorporating paternal factors into fertility assessments.

Keywords: IVF, clinical pregnancy, live birth, male age, AMH

Introduction

Assisted reproductive technology (ART), encompassing techniques such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) followed by embryo transfer (collectively termed IVF/ICSI-ET), has revolutionized infertility treatment and enabled many couples to achieve parenthood. Despite significant technological advancements in ovarian stimulation, embryo culture, and transfer techniques, the success rates of IVF/ICSI-ET remain modest, with global clinical pregnancy rates ranging between 30–40% per cycle and live birth rates slightly lower.[1,2] Identifying factors that influence these outcomes remains a top priority to improve clinical decision-making and tailor treatment protocols.

Among known prognostic indicators, female age is the most consistently recognized determinant of IVF/ICSI-ET success, largely due to its impact on oocyte quality and chromosomal competence.[3] Other female factors, such as anti-Müllerian hormone (AMH) levels, antral follicle count, and baseline follicle-stimulating hormone (FSH), have also been extensively studied and incorporated into predictive models.[4] Less attention, however, has been paid to male-related factors, despite growing evidence that paternal age and sperm quality may influence fertilization, embryo development, and ultimately implantation and live birth.[5,6] Several recent studies demonstrate significantly higher clinical pregnancy and live birth rates among men under 40.[7,8] However, others report no association between male age and ART outcomes.[6]

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Moreover, laboratory and treatment-related variables—including fertilization method (IVF vs. ICSI), embryo quality, and number of embryos transferred—also influence IVF/ICSI-ET outcomes. Yet most existing research tends to assess these factors separately, without simultaneously integrating clinical variables, hormonal profiles, embryologic metrics, and treatment protocols in multivariate models.[9,10] Therefore, a holistic analysis integrating both clinical and laboratory associations is essential for individualized treatment and accurate counseling.

Based on the above evidence, we hypothesized that both male-related factors (particularly paternal age) and female hormonal markers (such as AMH) would independently contribute to IVF/ICSI-ET outcomes, and that their effects would remain significant even after controlling for embryologic and treatment variables. The variables selected in this study — including male and female age, AMH, gonadotropin dosage, embryo quality, and number of embryos transferred — were chosen based on their established or emerging clinical relevance in the ART literature, as well as their routine availability in our clinical database. This study aims to investigate the associations between these selected clinical, hormonal, and embryologic parameters and IVF/ICSI-ET outcomes, specifically clinical pregnancy and live birth, with the goal of identifying independently associated factors to support individualized, evidence-based clinical decision-making.

Materials and Methods

Study Design and Population

This retrospective cohort study was undertaken at the Reproductive Medicine Center of [The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University], analyzing clinical and laboratory data from couples undergoing IVF/ICSI-ET cycles between January 2020 and December 2024. Ethical approval was obtained from the Institutional Review Board of The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University (Approval No. KY-2025-221-01), and all patient data were anonymized to ensure confidentiality. This study was conducted in accordance with the Declaration of Helsinki. Retrospective data collection exempted this study from obtaining informed consent.

Inclusion criteria were as follows: (1) Use of autologous oocytes and partner's sperm; (2) Complete records on clinical, hormonal, and embryological variables. To ensure the statistical independence of observations and avoid within-couple clustering effects, only the first eligible IVF/ICSI-ET cycle per couple within the study period was included in the final analysis. Couples who underwent more than one cycle during January 2020 to December 2024 contributed only their initial qualifying cycle. The unit of analysis was therefore the IVF/ICSI-ET cycle, with a maximum of one cycle per couple.

Exclusion criteria included: (1) Use of donor sperm or donor oocytes; (2) Presence of uterine abnormalities (eg, fibroids, intrauterine adhesions) affecting implantation; (3) Severe oligozoospermia or azoospermia requiring surgical sperm retrieval; (4) Preimplantation genetic testing (PGT) cycles.

The exclusion criteria were pre-specified prior to data extraction and applied systematically to all cases in order to minimize selection bias. The final eligible sample of 139 cycles reflects strict quality control of clinical data rather than selective inclusion. To ensure independence of observations, only the first eligible IVF/ICSI-ET cycle per couple within the study period was included in the analysis. Couples with multiple cycles during the study period had only their initial qualifying cycle analyzed. The unit of analysis was therefore the IVF/ICSI-ET cycle, with one cycle per couple.

Controlled Ovarian Stimulation and Embryology Procedures

Ovarian stimulation was initiated using a GnRH agonist long protocol. The choice of downregulation agent included triptorelin, leuprorelin, or goserelin, administered based on physician discretion. Downregulation was typically started in the mid-luteal phase of the preceding cycle, and its duration (in days) and dose were recorded.

Following adequate pituitary suppression (E2 <50 pg/mL, LH <5 mIU/mL), controlled ovarian stimulation commenced using recombinant FSH and/or human menopausal gonadotropin (HMG), with individualized dosing based on age, BMI, and baseline ovarian reserve (AMH, AFC). Adequate pituitary downregulation was confirmed by serum E2 < 50 pg/mL, LH < 5 mIU/mL, and endometrial thickness < 5 mm on ultrasound.

Ovulation was triggered with 250 μg recombinant hCG when at least two leading follicles reached ≥18 mm. Oocyte retrieval was performed 34–36 hours post-trigger. The number of follicles >14 mm, aspirated follicles, and retrieved oocytes were documented. Mature oocytes were fertilized via: IVF, ICSI, or IVF followed by rescue ICSI.

Embryo Culture, Assessment, and Transfer

Embryo development was monitored in vitro: Fertilization rate was calculated as the number of two-pronuclei (2PN) zygotes divided by total retrieved oocytes. High-quality embryo rate was defined by morphological scoring on Day 3 or blastocyst grading on Day 5 using standard criteria. Embryo transfer was performed on Day 3 or Day 5, based on embryo quality and clinical decision. The number of embryos transferred (1 or 2) was recorded. Luteal phase support was initiated post-retrieval with vaginal or intramuscular progesterone. On Day 3, high-quality embryos were defined as those with 7–9 equally sized blastomeres, < 10% fragmentation, and no multinucleation. On Day 5, high-quality blastocysts were defined as those with an expansion grade of ≥3, inner cell mass grade of A or B, and trophectoderm grade of A or B, according to the Gardner grading system.

Outcome Measures

Primary Outcomes Included

Clinical pregnancy was defined as the ultrasound-confirmed presence of at least one intrauterine gestational sac with fetal cardiac activity at 6–7 weeks of gestation. Live birth was defined as the delivery of at least one live neonate at or beyond 24 completed weeks of gestation. These definitions are consistent with the revised glossary of ART terminology published by the International Committee for Monitoring Assisted Reproductive Technologies (ICMART).

Independent Variables Collected

A comprehensive set of variables was extracted for each patient. Demographics: female and male age, female BMI. Baseline endocrine profile: FSH, LH, E2, P, T, AMH. Stimulation parameters: Gn duration, total Gn dose, HMG dose, recombinant FSH dose, initial dose. Downregulation details: agent used, initiation day, duration. Hormonal profile on stimulation start and hCG day: serum E2, LH, FSH, P. Follicle and oocyte parameters: number of follicles ≥14 mm, aspirated follicles, retrieved oocytes, high-quality oocytes. Embryology indicators: fertilization rate, high-quality embryo rate. Endometrial thickness: measured on the day of hCG. Fertilization method: IVF, ICSI, or rescue ICSI. Embryo transfer stage was categorized as Day 3 (cleavage-stage) or Day 5 (blastocyst-stage) transfer, and this variable was included in the univariate and multivariable analyses. Where available, basic semen parameters, including sperm concentration, progressive motility, and normal morphology (assessed according to WHO 2010 criteria), were extracted from laboratory records and included in the analysis as additional male factor covariates.

Statistical Analysis

Data were analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY). Continuous data are reported as mean ± standard deviation (SD) when normally distributed; otherwise as median (25th–75th percentiles) depending on distribution, and compared using Student's *t*-test or Mann–Whitney *U*-test. Categorical variables were presented as frequency and compared using the Chi-square or Fisher's exact test.

Potential associations underwent univariate logistic regression analysis. Variables with a p-value <0.10 were entered into multivariable logistic regression models to identify independently associated factors. Odds ratios (ORs) and 95% confidence intervals (CIs) were reported. Model performance was evaluated by area under the receiver operating characteristic curve (AUC). p <0.05 was considered statistically significant. To assess the adequacy of statistical power, a post-hoc power analysis was performed using G*Power (version 3.1).

Results

Data Overview

A total of 178 cases were initially considered for the study, with 39 cases excluded due to various reasons, such as failure to undergo embryo transfer or incomplete data. This left 139 cases eligible for analysis, of which 72 achieved clinical pregnancy and 67 did not. The clinical pregnancy rate was calculated as 72/139, which is approximately 51.8%. Among the 72 pregnancies, 53 resulted in live births, while 19 resulted in non-live births (miscarriage or other complications), the live birth rate was 53/139, or approximately 38.1% (Figure 1).

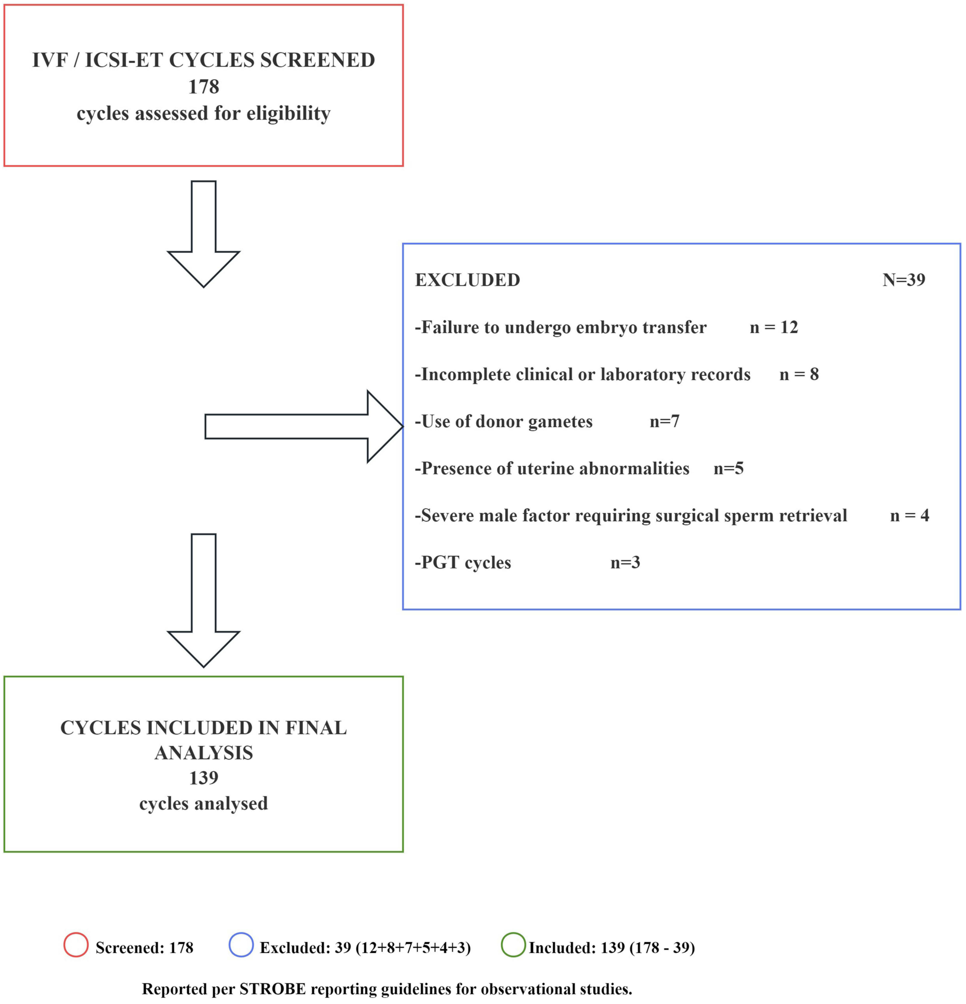

Figure 1 Study Flowchart.

Comparison of Baseline Characteristics Between Pregnancy and Non-Pregnancy Groups

The analysis of baseline characteristics revealed several significant differences between the pregnancy and non-pregnancy groups (Table 1). Male age was significantly younger in the pregnancy group (30.500 ± 3.568 years) compared to the non-pregnancy group (32.493 ± 4.695 years) with a p-value of 0.005. Basal AMH levels were also significantly lower in the pregnancy group compared to the non-pregnancy group (2.85 (2.08, 4.928) vs 3.68 (2.69, 5.28), $p = 0.031$). Infertility duration showed a notable difference, with a higher proportion of patients in the pregnancy group having infertility for less than 3 years (57 vs. 40, $p = 0.013$). The pregnancy group also had a higher proportion of patients who underwent transfer of two embryos (48 vs. 33, $p = 0.037$). Other factors, including female age, BMI, GnRH days, total Gn dose, oocyte retrieval number, fertilization rates, embryo quality, the types of infertility (primary or secondary), the method of fertilization (IVF, ICSI, or IVF + Rescue ICSI), or the suppression medication used (Triptorelin, Leuprorelin, Goserelin) did not show significant differences between the two groups.

Binary Logistic Regression Analysis Results

To identify factors influencing pregnancy success, we performed a binary logistic regression analysis. Initially, variables with $p < 0.05$ in the univariate analysis were included in the regression model (Table 2). The results revealed that male age (OR (95% CI): 0.878 (0.797, 0.967), $p = 0.008$) and basal AMH (OR (95% CI): 0.829 (0.691, 0.995), $p = 0.044$)

Table 1 Comparison of Baseline Characteristics Between Pregnancy and Non-Pregnancy Groups

Variable	Non-Pregnancy Group	Pregnancy Group	Statistical Value (t/z/Chi-Square)	p-value
Female Age (years)	30.239 ± 4.079	29.653 ± 3.682	0.890	0.375
Male Age (years)	32.493 ± 4.695	30.500 ± 3.568	2.829	0.005
Female BMI (kg/m^2)	23.256 ± 3.705	22.542 ± 3.302	1.202	0.231
Gn Days	11 (10, 12)	12 (10, 13)	−1.063	0.288
Gn Total Dose (IU)	1987.5 (1650, 2475)	2106.25 (1800, 2793.75)	−1.223	0.221
Follicles ≥14 mm on HCG Day	9.179 ± 4.379	9.653 ± 4.095	−0.659	0.511
Number of Oocytes Retrieved	13.522 ± 5.498	12.500 ± 5.397	1.106	0.271
Number of Oocytes Retrieved (MII)	10.075 ± 4.564	9.778 ± 3.784	0.419	0.676
Number of High-Quality Oocytes	8 (6, 11)	8 (6, 10.75)	−0.341	0.733
Fertilization Rate	0.637 ± 0.188	0.695 ± 0.201	−1.748	0.083
High-Quality Embryo Rate	0.330 (0, 0.500)	0.400 (0.228, 0.670)	−1.843	0.065
Basal FSH (mIU/mL)	6.26 (5.16, 7.23)	6.64 (5.495, 7.938)	−1.345	0.179
Embryo Transfer Day				
Day 3 n (%)	45 (67.2)	48 (66.7)	$\chi^2 = 0.003$	0.954
Day 5 n (%)	22 (32.8)	24 (33.3)		
Transfer type				
Fresh, n (%)	60 (89.6)	65 (90.3)	$\chi^2 = 0.018$	0.893
Frozen, n (%)	7 (10.4)	7 (9.7)		

Notes: Negative t or z values indicate that the mean or median of the variable was higher in the pregnancy group than in the non-pregnancy group. Continuous variables are presented as mean ± SD for normally distributed data or median (25th–75th percentile) for non-normally distributed data, as determined by the Shapiro–Wilk test. Normality assessment results are available upon request.

Table 2 Single Factor Binary Logistic Regression Analysis of Clinical Pregnancy (Including Single Factor $p < 0.05$ Variables)

Variable	B	Standard Error	Wald	p-value	OR	OR 95% CI
Male Age	−0.13	0.049	7.012	0.008	0.878	0.797, 0.967
Basal AMH (ng/mL)	−0.187	0.093	4.05	0.044	0.829	0.691, 0.995
Duration of Infertility (years)	0.075	0.371	0.041	0.84	1.078	0.521, 2.228
Number of Embryos Transferred	0.554	0.372	2.225	0.136	1.741	0.840, 3.605

Notes: Categorical variables are presented with their reference category noted. Continuous variables represent the OR per one-unit increase.

Abbreviations: B, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval.

were significantly associated with pregnancy outcomes. Specifically, younger male age and lower basal AMH levels were found to be predictive of pregnancy success. However, neither the duration of infertility nor the number of embryos transferred reached statistical significance ($p = 0.84$ and $p = 0.136$, respectively).

In the second analysis, we included additional factors identified from the univariate analysis with $p < 0.1$ along with female age (Table 3). The results showed that male age remained a significant association (OR (95% CI): 0.85 (0.743, 0.972), $p = 0.017$), while basal AMH was not significant in this model ($p = 0.18$). Additionally, the number of embryos transferred ($p = 0.097$), the fertilization rate ($p = 0.163$) and high-quality embryo rate ($p = 0.192$) did not show significant discriminative abilityfor pregnancy success, and female age was not a significant factor ($p = 0.325$). It should be noted that the confidence intervals for Fertilization Rate (OR 3.886, 95% CI: 0.578–26.131) and High-Quality Embryo Rate (OR 2.182, 95% CI: 0.676–7.047) are extremely wide, indicating that these estimates are statistically unstable and should be interpreted with considerable caution. This instability is attributable to the limited sample size and the relatively small number of outcome events, which reduces the reliability of the regression model for these variables.

Binary logistic regression analysis showed that male age was an independent risk factor for successful pregnancy. ROC curve analysis was conducted in an exploratory capacity to identify a potential threshold for male age (Figure 2). The AUC was 0.620 (95% CI: 0.527–0.713), indicating only modest discriminative ability. A data-derived cut-off of 31.5 years was

Table 3 Multivariate Binary Logistic Regression Analysis of Clinical Pregnancy (Including Univariate p < 0.1 and Female Age Variables)

Variable	B	Standard Error	Wald	p-value	OR	OR 95% CI
Male Age	−0.163	0.068	5.652	0.017	0.85	0.743, 0.972
Basal AMH (ng/mL)	−0.127	0.095	1.794	0.180	0.881	0.732, 1.06
Duration of Infertility (years)	0.134	0.382	0.124	0.725	1.144	0.541, 2.417
Number of Embryos Transferred	0.639	0.385	2.75	0.097	1.894	0.89, 4.029
Fertilization Rate	1.357	0.972	1.948	0.163	3.886	0.578, 26.131
High-Quality Embryo Rate	0.78	0.598	1.702	0.192	2.182	0.676, 7.047
Female Age	0.067	0.069	0.969	0.325	1.07	0.935, 1.224

Notes: Categorical variables are presented with their reference category noted. Continuous variables represent the OR per one-unit increase.

Abbreviations: B, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval.

identified; however, given the limited sample size and the low AUC value, this threshold should not be interpreted as a clinically validated criterion and requires confirmation in independent, larger cohorts before any clinical application.

Comparison of Factors Between Live Birth and Non-Live Birth Groups

Among the 72 clinical pregnancies, 53 resulted in live births and 19 in non-live births. Univariate analysis revealed several significant differences between the live birth and non-live birth groups (Table 4). The live birth group had significantly younger female age (median 29 vs. 32 years; p = 0.014) and male age (29.96 ± 3.34 vs. 32.00 ± 3.84 years; p = 0.032). Additionally, the total HMG dose was notably lower in the live birth group (median 525 IU vs. 900 IU; p = 0.003), as was the Gn start dose (175 (143.75, 200) vs. 178.947 (178.947, 200); p = 0.034). A significant difference was

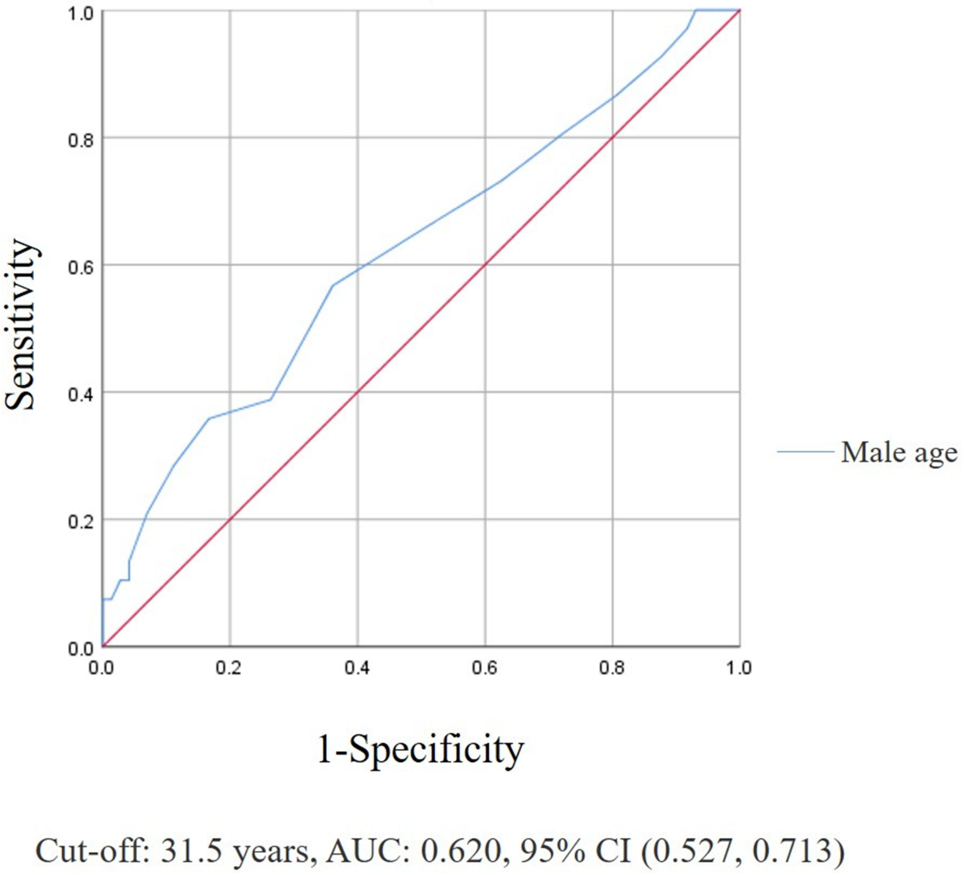

Figure 2 ROC curve for predicting clinical pregnancy in IVF/ICSI-ET cycles based on male age.

Table 4 Comparison of Factors Between Live Birth and Non-Live Birth Groups

Variable	Live Birth Group (n=53)	Non-Live Birth Group (n=19)	t/z/Chi-Square	p-value
Female Age (years)	29 (28, 31)	32 (29.5, 33.5)	−2.467	0.014
Male Age (years)	29.962 ± 3.340	32 ± 3.844	−2.192	0.032
Female BMI (kg/m^2)	21.8 (20.3, 24.8)	22.1 (19.75, 25.8)	−0.128	0.898
Gn Days	12 (10, 13)	12 (10, 12.5)	−0.220	0.826
Gn Total Dose (IU)	2100 (1750, 2487.5)	2225 (1800, 3037.5)	−0.741	0.458
Follicles ≥14 mm on HCG Day	9.660 ± 4.165	9.632 ± 4.003	0.026	0.979
Number of Oocytes Retrieved	12.566 ± 5.224	12.316 ± 6.000	0.172	0.864
Number of Oocytes Retrieved (MII)	9.642 ± 3.727	10.158 ± 4.018	−0.508	0.613
Number of High-Quality Oocytes	8.283 ± 3.066	8.111 ± 3.428	0.199	0.842
Fertilization Rate	0.670 (0.560, 0.800)	0.750 (0.520, 0.890)	−1.107	0.268
High-Quality Embryo Rate	0.500 (0.290, 0.750)	0.400 (0.200, 0.670)	−0.982	0.326
Basal FSH (mIU/mL)	6.56 (5.62, 7.72)	6.94 (4.365, 8.165)	−0.294	0.769
Embryo Transfer Day				
Day 3	35 (66.0)	13 (68.4)	χ^2 = 0.037	0.847
Day 5	18 (34.0)	6 (31.6)		
Transfer type				
Fresh, n (%)	48 (90.6)	17 (89.5)	χ^2 = 0.018	0.893
Frozen, n (%)	5 (9.4)	2 (10.5)		

Table 5 Multivariate Binary Logistic Regression Analysis of Live Births

Variable	B	Standard Error	Wald	p-value	OR	OR 95% CI
Female Age	−0.131	0.126	1.078	0.299	0.877	0.685, 1.123
Male Age	−0.061	0.126	0.236	0.627	0.94	0.734, 1.205
Total HMG Dose	−0.001	0.001	3.452	0.063	0.999	0.997, 1
Number of Embryos Transferred	0.708	0.66	1.151	0.283	2.031	0.557, 7.406
Gn Start Dose	−0.01	0.008	1.703	0.192	0.99	0.975, 1.005

Notes: Categorical variables are presented with their reference category noted. Continuous variables represent the OR per one-unit increase.

Abbreviations: B, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval.

also observed in the number of embryos transferred, with a higher proportion of double-embryo transfers in the live birth group (39 vs. 9; p = 0.038). However, multivariate logistic regression analysis (Table 5) showed that none of these variables were independently associated with live birth outcomes. Although total HMG dose approached significance (p = 0.063), variables such as female age (p = 0.299), male age (p = 0.627), Gn start dose (p = 0.192), and number of embryos transferred (p = 0.283) did not reach statistical significance in the adjusted model.

Discussion

In this retrospective study of IVF/ICSI-ET cycles, we examined a broad set of clinical, hormonal, and embryologic factors to identify potential associations of clinical pregnancy and live birth. Our findings indicate that several variables—such as male age, female age, AMH levels, gonadotropin doses, and the number of embryos transferred—were associated with reproductive outcomes in univariate analyses. However, after adjusting for confounding factors in multivariate models, only male age emerged as an independently associated factor of clinical pregnancy, while no single variable was independently predictive of live birth. These results suggest that while individual factors may appear influential in isolation, IVF/ICSI-ET success is ultimately shaped by a complex interplay of male, female, and treatment-related parameters.

Our analysis confirms male age as a significant factor in determining clinical pregnancy outcomes in IVF/ICSI-ET, with younger male age being associated with higher pregnancy success rates. The results are in agreement with several

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recent studies that underscore the importance of male age in ART outcomes.[11,12] Although our study did not directly measure sperm DNA quality, the existing literature suggests that male age may exert its influence on ART outcomes through progressive deterioration of sperm DNA integrity and chromosomal competence.[13,14] Our findings are consistent with this hypothesis, though direct mechanistic confirmation requires prospective studies incorporating sperm DNA fragmentation analysis.[13,14] In our cohort, male age was identified as an independently associated factor of clinical pregnancy in both univariate and multivariate logistic regression models (OR: 0.878, p=0.008 and OR: 0.85, p=0.017, respectively). The cut-off for male age was identified as 31.5 years, with an AUC of 0.620 (95% CI: 0.527–0.713) in ROC curve analysis, suggesting that male age can be considered a moderate association for IVF/ICSI-ET success. However, it is important to note that this cut-off of 31.5 years is substantially lower than the thresholds commonly reported in the paternal age literature, where adverse effects on ART outcomes are generally discussed in the context of men aged ≥40 or ≥45 years [refs]. A cut-off as young as 31.5 years has limited biological plausibility, as meaningful differences in sperm DNA fragmentation between men in their early thirties are unlikely to exert a dramatic clinical impact. Therefore, this threshold should be interpreted with caution: it may represent statistical overfitting to the relatively small sample in the present study, rather than reflecting a true biological boundary. The clinical validity of this cut-off requires confirmation in larger, independent cohorts before it can be applied in clinical practice. It is important to note that, while male age was found to be a significant association of clinical pregnancy, it was not identified as an independently associated factor of live birth in our cohort. This suggests that while younger male age may increase the likelihood of successful implantation and early pregnancy, it does not necessarily translate to improved live birth rates. Those finding contrasts with some previous studies that have reported no direct relationship between male age and reproductive outcomes in ART. For instance, several studies have suggested that, despite the well-documented decline in sperm quality with advancing paternal age, male age does not significantly impact either clinical pregnancy rates or live birth rates.[15,16]

Our study contributes to the growing body of literature on male age and ART outcomes, highlighting its importance in clinical pregnancy prediction. However, the lack of association with live birth underlines the complexity of ART outcomes, where multiple variables interact to determine success. An important limitation of the current analysis is that sperm quality parameters, including sperm concentration, progressive motility, and normal morphology, were not incorporated into the regression models, primarily due to incomplete documentation of these values across all cycles in our retrospective database. Since advancing paternal age is well known to be associated with declining sperm quality and increased sperm DNA fragmentation, it is possible that part of the observed effect of male age on clinical pregnancy is mediated through, or confounded by, underlying sperm quality. Consequently, the characterization of male age as an "independent" association should be interpreted with appropriate caution: it reflects independence from the clinical and embryologic variables included in our models, but does not exclude the possibility that sperm parameters represent the true mechanistic driver. Future studies should incorporate comprehensive semen analysis data, including sperm DNA fragmentation indices, alongside male age in multivariate models to more rigorously establish the independent contribution of paternal age to IVF/ICSI-ET outcomes. Further prospective studies that examine male-related factors, including sperm DNA quality and other clinical and embryological variables, are necessary to refine our understanding of the role of male age in IVF/ICSI-ET.

Female age has long been recognized as a major determinant of IVF/ICSI-ET success, given its established effects on ovarian reserve, oocyte quality, and embryo development. However, in our cohort, female age did not show a statistically significant difference between the pregnancy and non-pregnancy groups (p>0.05), although it was significantly associated with live birth outcomes in univariate analysis (p<0.05). Nevertheless, female age did not remain an independently associated factor of live birth in the multivariate logistic regression model. This suggests that while age may influence aspects of reproductive potential, it does not independently predict IVF/ICSI-ET success when other variables are taken into account. This finding diverges from the commonly reported pattern in ART literature, where female age is typically considered one of the strongest associations of IVF/ICSI-ET outcomes.[17–19]

We also assessed AMH, a commonly used marker of ovarian reserve. In our study, AMH levels were significantly different between the clinical pregnancy and non-pregnancy groups (2.85 ng/mL vs. 3.68 ng/mL, p=0.031), with lower AMH observed in the pregnancy group. However, similar to female age, AMH did not retain statistical significance in the multivariate model. This result indicates that while AMH may reflect aspects of ovarian response, its discriminative abilityfor clinical pregnancy is limited when controlling for other confounding factors. This observation is in line with recent studies suggesting that AMH alone may not be sufficient for predicting IVF/ICSI-ET success, particularly in patient populations without markedly diminished ovarian reserve.[20,21] The paradoxical finding of lower AMH in the clinical pregnancy group (2.85 vs. 3.68 ng/mL) deserves careful consideration. In the general ART population, higher AMH is associated with greater ovarian reserve, a higher number of retrieved oocytes, and, generally, improved pregnancy outcomes [ref]. However, this relationship is not uniformly linear and may be confounded by the presence of polycystic ovary syndrome (PCOS). Women with PCOS characteristically exhibit elevated AMH levels, often two to four times higher than age-matched controls, yet may paradoxically experience poorer oocyte quality, higher rates of cycle cancellation due to OHSS risk, and suboptimal embryo development [ref]. In our cohort, although patients with clinically diagnosed PCOS were not explicitly excluded, the possibility that a subset of women in the non-pregnancy group had undiagnosed or inadequately managed PCOS — reflected by higher AMH values (median 3.68 ng/mL) — cannot be excluded. Such patients may have contributed elevated AMH values to the non-pregnancy group while demonstrating poorer embryo quality or implantation potential. Unfortunately, the retrospective design of this study precluded a formal PCOS subgroup analysis, as detailed hormonal and ultrasound criteria were not uniformly documented for all patients. This represents an important limitation, and future studies should specifically examine the distribution of AMH levels stratified by PCOS status, oocyte quality metrics, and fertilization outcomes in order to clarify this apparently paradoxical relationship. The exclusion or stratification of PCOS patients in future analyses will be critical for interpreting the true discriminative abilityof AMH in this population.

Taken together, our findings reinforce the idea that neither female age nor AMH should be interpreted in isolation when counseling patients about IVF/ICSI-ET prognosis. While these factors provide useful information regarding ovarian biology, their impact on IVF/ICSI-ET outcomes is influenced by a broader set of variables, including embryo quality, endometrial receptivity, and male factors. This underscores the importance of a comprehensive, multifactorial assessment in reproductive medicine.[22]

Embryo quality, fertilization method, and number of embryos transferred are key laboratory factors influencing IVF/ICSI-ET outcomes. In our study, no significant differences were observed in fertilization rate, high-quality embryo rate, or embryo transfer strategy between the pregnancy and non-pregnancy groups, diverging from previous studies where these factors were predictive of success.[22,23] This may reflect the homogeneity of laboratory protocols or limited subgroup sizes, particularly among patients undergoing single embryo transfer. However, a higher proportion of two-embryo transfers was noted in the pregnancy group (48 vs. 33, p=0.037), suggesting a potential benefit in select patients. Although not a significant association in multivariate analysis, this finding aligns with clinical practices where multiple embryos may be transferred in cases with poor prognosis. Ovarian stimulation variables, including type and dosage of gonadotropins, showed no significant association with clinical pregnancy in our cohort. However, the live birth group had significantly lower total HMG doses and Gn starting doses (p=0.003 and p=0.032), potentially indicating a more favorable ovarian response or effective protocol optimization. While the ideal gonadotropin dosage remains controversial, individualized stimulation strategies based on ovarian reserve markers (eg, AMH, AFC) are increasingly advocated.[24] Our findings support further refinement of stimulation protocols to balance efficacy and safety, particularly in minimizing the risk of OHSS.

Limitations and Future Directions

This study has several limitations that should be considered when interpreting the results. First, the retrospective nature of the analysis introduces the potential for selection bias, particularly in the exclusion of patients with incomplete data or those who failed to undergo embryo transfer. Although the exclusion of 39 cases from the initial 178 was based on pre-defined clinical criteria (incomplete records, failed embryo transfer, use of donor gametes, etc)., we acknowledge that this process may nonetheless introduce a degree of selection bias, as patients with complete data may systematically differ from those excluded. Furthermore, the relatively low case volume over a five-year single-center period may reflect the application of strict eligibility criteria, but also limits the generalizability of our findings. Readers should therefore exercise caution in extrapolating these results to broader clinical populations, and independent validation in larger, multi-center cohorts is strongly encouraged before any clinical implementation of the identified associations. Second, the sample size of the study is a critical limitation, particularly for the live birth analysis where the non-live birth subgroup comprised only 19 cases. Post-hoc power analysis confirmed that the study was underpowered (achieved power ≈ 0.30–0.35) for the live birth multivariate model. Consequently, the absence of statistically significant independently associated factors in Table 5 should be interpreted with caution, as this may reflect a Type II error — failure to detect true effects due to inadequate sample size — rather than a genuine absence of association. The non-significant trends observed (eg, total HMG dose, p = 0.063) may have reached significance in a larger cohort. Future multi-center prospective studies with larger sample sizes are needed to confirm or refute these findings. A larger, multi-center prospective study would provide a more robust dataset for generalizing findings to a broader population. Additionally, we focused primarily on clinical and treatment-related factors, without accounting for other variables such as genetic factors, lifestyle influences, and patient comorbidities that may also impact IVF/ICSI-ET success. Future research should seek to integrate these additional factors into predictive models to provide a more holistic view of IVF/ICSI-ET prognosis. Furthermore, sperm quality parameters (concentration, motility, morphology, and DNA fragmentation) were not included in the regression models due to incomplete data availability in our retrospective cohort. This represents a meaningful gap, as sperm quality is a plausible mediator of the male age effect. Future prospective studies should systematically collect and incorporate these variables. Furthermore, due to the retrospective observational design, unmeasured confounders, including lifestyle factors, genetic background, endometrial receptivity markers, and sperm DNA quality, may have influenced the observed associations. No causal inferences should therefore be drawn from these findings.

Conclusion

In this retrospective cohort study, younger male age was consistently associated with higher clinical pregnancy rates in IVF/ICSI-ET cycles, emerging as an independently associated factor in multivariate logistic regression. These findings highlight the importance of incorporating paternal factors into fertility assessments and pre-treatment counseling. However, several important caveats must be acknowledged. The male age cut-off of 31.5 years derived from ROC analysis requires cautious interpretation, as it may reflect statistical characteristics of this small, single-center cohort rather than a broadly applicable biological threshold. The paradoxical finding of lower AMH in the clinical pregnancy group warrants further investigation, particularly in relation to the potential confounding role of undiagnosed PCOS. The absence of sperm quality parameters from our regression models also limits the strength of conclusions regarding male age as a truly independent factor. No independently associated factor of live birth was identified, likely reflecting insufficient statistical power given the limited subgroup sizes. A comprehensive, individualized approach that integrates both male and female clinical, hormonal, and laboratory parameters remains essential for optimizing ART outcomes. Larger, prospective, multi-center studies incorporating sperm quality metrics and PCOS stratification are needed to validate and extend these findings.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

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Disclosure

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References

1. Wyns C, Bergh C, Strohmer H, et al; European IVF-monitoring Consortium (EIM)‡ for the European Society of Human Reproduction and Embryology (ESHRE). ART in Europe, 2016: results generated from European registries by ESHRE. *Hum Reprod Open*. 2020;2020(3):hoaa032. doi:10.1093/hropen/hoaa032
2. SART. Assisted reproductive technology national summary report. 2022. Available from: https://www.sart.org. Accessed March 25, 2026.
3. Broekmans FJ, Knauff EA, te Velde ER, Macklon NS, Fauser BC. Female reproductive ageing: current knowledge and future trends. *Trends Endocrinol Metab*. 2007;18(2):58–65. doi:10.1016/j.tem.2007.01.004

4. La Marca A, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: from theory to practice. *Hum Reprod Update*. 2014;20(1):124–140. doi:10.1093/humupd/dmt037
5. Robinson L, Gallos ID, Conner SJ, et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. *Hum Reprod*. 2012;27(10):2908–2917. PMID: 22791753. doi:10.1093/humrep/des261
6. Kasman AM, Li S, Zhao Q, Behr B, Eisenberg ML. Relationship between male age, semen parameters and assisted reproductive technology outcomes. *Andrology*. 2021;9(1):245–252. doi:10.1111/andr.12908
7. du Fossé NA, van der Hoorn MP, Van Lith JMM, Le Cessie S, Lashley EELO. Advanced paternal age is associated with an increased risk of spontaneous miscarriage: a systematic review and meta-analysis. *Hum Reprod Update*. 2020;26(5):650–669. doi:10.1093/humupd/dmaa010
8. Murugesu S, Kasaven LS, Petrie A, et al. Does advanced paternal age affect outcomes following assisted reproductive technology? A systematic review and meta-analysis. *Reprod Biomed Online*. 2022;45(2):283–331. doi:10.1016/j.rbmo.2022.03.031
9. Coban O, Serdarogullari M, Pervaiz R, Soykok A, Yarkiner Z, Bankeroglu H. Effect of paternal age on assisted reproductive outcomes in ICSI donor cycles. *Andrology*. 2023;11(3):515–522. doi:10.1111/andr.13363
10. Liu H, Zhao H, Yu G, et al. Conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI): which is preferred for advanced age patients with five or fewer oocytes retrieved? *Arch Gynecol Obstet*. 2018;297(5):1301–1306. doi:10.1007/s00404-018-4696-6
11. Gourinat A, Mazeaud C, Hubert J, Eschwege P, Koscinski I. Impact of paternal age on assisted reproductive technology outcomes and offspring health: a systematic review. *Andrology*. 2023;11(6):973–986. doi:10.1111/andr.13385
12. Kaltsas A, Zikopoulos A, Vrachnis D, et al. Advanced paternal age in focus: unraveling its influence on assisted reproductive technology outcomes. *J Clin Med*. 2024;13(10):2731. doi:10.3390/jcm13102731
13. Almeida S, Rato L, Sousa M, Alves MG, Oliveira PF. Fertility and sperm quality in the aging male. *Curr Pharm Des*. 2017;23(30):4429–4437. doi:10.2174/1381612823666170503150313
14. Albani E, Castellano S, Gurrieri B, et al. Male age: negative impact on sperm DNA fragmentation. *Aging*. 2019;11(9):2749–2761. doi:10.18632/aging.101946
15. Xie H, Chen Y, Xu S, et al. Increasing age in men is negatively associated with sperm quality and DNA integrity but not pregnancy outcomes in assisted reproductive technology. *Front Aging*. 2025;6:1603916. doi:10.3389/fragi.2025.1603916
16. Beguería R, García D, Obradors A, Poisot F, Vassena R, Vernaeve V. Paternal age and assisted reproductive outcomes in ICSI donor oocytes: is there an effect of older fathers? *Hum Reprod*. 2014;29(10):2114–2122. doi:10.1093/humrep/deu189
17. Franasiak JM, Forman EJ, Hong KH, et al. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. *Fertil Steril*. 2014;101(3):656–663.e1. doi:10.1016/j.fertnstert.2013.11.004
18. Balachandren N, Salman M, Diu NL, Schwab S, Rajah K, Mavrelos D. Ovarian reserve as a predictor of cumulative live birth. *Eur J Obstet Gynecol Reprod Biol*. 2020;252:273–277. doi:10.1016/j.ejogrb.2020.06.063
19. Qu P, Chen L, Zhao D, Shi W, Shi J. Nomogram for the cumulative live birth in women undergoing the first IVF cycle: base on 26, 689 patients in China. *Front Endocrinol*. 2022;13:900829. doi:10.3389/fendo.2022.900829
20. Li HWR, Nelson SM. Clinical application of AMH measurement in assisted reproduction. *Front Endocrinol*. 2020;11:606744. doi:10.3389/fendo.2020.606744
21. Hou Y, Wang L, Li Y, Ai J, Tian L. Serum levels of anti-Müllerian hormone influence pregnancy outcomes associated with gonadotropin-releasing hormone antagonist treatment: a retrospective cohort study. *Sci Rep*. 2023;13(1):2127. doi:10.1038/s41598-023-28724-8
22. Shingshetty L, Cameron NJ, Mclernon DJ, Bhattacharya S. Predictors of success after in vitro fertilization. *Fertil Steril*. 2024;121(5):742–751. doi:10.1016/j.fertnstert.2024.03.003
23. Jia Y, Ai Z, Zhu X, et al. Analysis of predictors of clinical pregnancy and live birth in patients with RIF treated with IVF-ET technology: a cohort study based on a propensity score approach. *Front Med Lausanne*. 2024;11:1348733. doi:10.3389/fmed.2024.1348733
24. Schouten N, Wang R, Torrance H, et al. Development and validation of a gonadotropin dose selection model for optimized ovarian stimulation in IVF/ICSI: an individual participant data meta-analysis. *Hum Reprod Update*. 2025;31(2):116–132. doi:10.1093/humupd/dmae032