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ASSISTED REPRODUCTION TECHNOLOGIES

Semen parameters on the day of oocyte retrieval predict low fertilization during conventional insemination IVF cycles

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Abstract

Purpose Poor fertilization during conventional IVF is difficult to predict in the absence of abnormal semen parameters; large-scale studies are lacking. The purpose of this study is to evaluate factors associated with low fertilization rates in conventional insemination IVF cycles.

Methods A retrospective cohort study evaluating demographic, reproductive evaluation, and IVF cycle characteristics to identify predictors of low fertilization (defined as 2PN/MII ≤ 30% per cycle). Participants were included if they were undergoing their first IVF cycle utilizing fresh autologous oocytes and conventional insemination with male partner's sperm (with normal pretreatment semen analysis). They were randomly divided into a training set and a validation set; validation modeling with logistic regression and binary distribution was utilized to identify covariates associated with low fertilization.

Results Postprocessing sperm concentration of less than 40 million/ml and postprocessing sperm motility ≤ 50% on the day of retrieval were the strongest predictors of low fertilization in the training dataset. Next, in the validation set, cycles with either low postprocessing concentration (≤ 40 million/ml) or low postprocessing progressive motility (≤ 50%) were 2.9–times (95% CI 1.4, 6.2) more likely to have low fertilization than cycles without either risk factor. Furthermore, cycles with low postprocessing concentration and progressive motility were 13.4 times (95% CI 4.01, 45.06) more likely to have low fertilization than cycles without either risk factor.

Conclusions Postprocessing concentration and progressive motility on the day of oocyte retrieval are predictive of low fertilization in conventional IVF cycles with normal pretreatment diagnostic semen analysis parameters.

Keywords In vitro fertilization · Conventional insemination · Assisted reproductive technologies · Semen analysis

Introduction

Prior to the introduction of intracytoplasmic sperm injection (ICSI) in the early 1990s, low fertilization and/or fertilization failure was anticipated in conventional insemination in vitro fertilization (IVF) cycles with known abnormal pretreatment semen analysis parameters [1, 2]. However, suboptimal fertilization or failed fertilization following conventional

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insemination is usually unpredictable in patients/couples with normal pretreatment semen parameters and risk factors for this outcome have not been confirmed. A low rate of normal fertilization (defined as the number of two pronuclear (2PN) zygotes/number of metaphase 2 (M2) oocytes of less than 30%) or absent fertilization complicates 5–20% of cycles with conventional insemination [3, 4]. Failed fertilization occurs in approximately 5–10% of IVF cycles with conventional insemination compared to 2–3% in IVF ICSI cycles [5, 6]. Since the estimated probability that a single mature oocyte will fertilize is approximately 30–40%, it is anticipated that the rate of fertilization failure would be directly related to the number of mature oocytes retrieved [3]. However, the incidence of fertilization failure is higher than would be expected based on these odds and warrants further investigation.

It has been suggested that gamete quality (oocytes and sperm, respectively) at the time of oocyte retrieval and insemination may play a role in mediating this low fertilization [7]. The inability of sperm to bind and/or penetrate the zona pellucida results in the absence of sperm nuclei in the unfertilized IVF oocyte [8, 9]. Unfortunately, there is a paucity of data investigating predictors for a normal rate of fertilization at the time of IVF, and it has proven difficult to predict the likelihood of low or no fertilization during conventional insemination among cycles with normal semen parameters. Given the utility of identifying those at risk for suboptimal fertilization during first IVF attempt with conventional insemination, and the potential benefit of offering ICSI preemptively, the objective of this study was to evaluate factors associated with low fertilization in autologous conventional insemination IVF cycles from couples without a diagnosis of male factor infertility.

Materials and methods

The study included couples that underwent controlled ovarian hyperstimulation and IVF at the Massachusetts General Hospital Fertility Center from 2004 through 2014. After institutional review board approval was obtained, data were derived from the electronic medical record system. The first IVF cycle attempt was exclusively examined to exclude any bias that prior cycles may confer on clinical management in subsequent cycles. Fresh IVF cycle attempts that utilized autologous gametes for IVF were reviewed ($N = 1{,}370$). Cycles which utilized ICSI, donor oocytes, pre-implantation genetic testing, had abnormal pretreatment diagnostic semen parameters (male factor infertility), or a Society for Assisted Reproductive Technology (SART) diagnosis of male factor infertility were excluded. Final analyses were limited to only cycles ($N = 663$) with complete information for ethnicity, age, body mass index (BMI), stimulation protocol, diagnosis, antral follicle counts, duration of infertility, ovulatory induction hormones levels, semen sample level, embryo transfers, and clinical outcomes. Patients with incomplete cycle or birth information were excluded. While it was anticipated that there would be female and male characteristics and diagnostic testing results that would be associated with the rate of normal fertilization, we had no a priori hypothesis on which specific tests would be related to this outcome.

Semen analysis assessment

Semen analyses were performed prior to beginning any treatment and again on the day of oocyte retrieval. Semen analyses were processed at Massachusetts General Hospital, as previously described [10]. Briefly, male patients were instructed to have between 2 and 3 days of abstinence prior to collection of the semen sample. After collection, the sample was allowed to liquefy for 15 minutes. The volume and viscosity were assessed using a wide-bore pipette. Semen aliquot was placed on a prewarmed Leja slide (Spectrum Technologies, CA). A computer-aided semen analyzer (CASA, HTM-IVOS Version 10HTM-IVOS, Beverly, MA, USA) was used to assess sperm concentration (million/ml) and motility (% motile). Motility was classified using World Health Organization (WHO) into progressive and non-progressive sperm cells [11]. Total progressive motile sperm count (million/ejaculate) was calculated by multiplying total sperm count by progressive motility. Sperm morphology was assessed was assessed with the Kruger strict criteria [12]. In addition to the evaluation above, the samples provided on the day of egg retrieval were processed by double-layer density gradient isolate (Irvine Scientific 99264, Santa Ana, CA). The sample was then washed twice to remove the isolate. The supernatant was removed, and the resulting pellet was resuspended in 1 ml of Quinn's Advantage Protein Plus Fertilization Medium (Sage 1520, Sage, Trumbull, CT).

Clinical protocols

Controlled ovarian hyperstimulation was performed with luteal-phase gonadotropin-releasing hormone (GnRH) agonist, GnRH-antagonist downregulation or GnRH agonist flare protocol, as clinically indicated [13]. Women were pretreated with oral contraceptive pills (OCPs) (30 μg ethinyl E_2/0.15 desogestrel, Apri, Teva Pharmaceuticals, North Wales, PA) or ethinyl estradiol patch (0.1 mg/day, Vivelle-Dot estradiol transdermal system, Novartis Pharmaceuticals Corporation, East Hanover, NJ) for follicular synchronization and/or priming. Pituitary downregulation with leuprolide acetate (Sandoz Inc., Princeton, NJ) was started 5 days before stopping OCPs in the by luteal-phase GnRH agonist cycle or 5 days after stopping OCPs in the GnRH agonist flare protocol. On the third day of induced menses, patients began controlled ovarian hyperstimulation with recombinant gonadotropins (Follistim,

Merck, White House Station, NJ; Gonal-F, EMD-Seron, Rockland, MA; and Menopur, Ferring Pharmaceuticals, Parsippany, NJ). Patients were serially monitored with serum estradiol and transvaginal ultrasound to assess follicular measurements and endometrial thickness. In the GnRH antagonist protocol, the GnRH antagonist (0.25 mg, ganirelix acetate, Organon, Roseland, NJ; or 0.25 mg, Cetrotide, EMD Serono, Rockland MA) was initiated when the serum estradiol concentration exceeded 1000 pg/ml or the lead follicle size reached 14 mm. When there were three lead follicles ≥ 16 mm, intramuscular human chorionic gonadotropin (hCG) (10,000 IU, Novarel, Ferring Pharmaceuticals or 10,000 IU, Pregnyl, Merck) was administered to induce final oocyte maturation. The patients underwent transvaginal ultrasound-guided oocyte retrieval 35–37 h after human chorionic gonadotropin trigger [13].

Following egg retrieval, oocytes underwent conventional insemination. Fertilization status of each oocyte was determined 24 h after insemination. Normal fertilization rate was defined as the ratio of the total fertilized oocytes with resultant 2 pronuclei zygotes (2PN) per the total number of MII phase matured oocytes inseminated. Treatment cycles were classified into two discrete categories based upon a threshold fertilization rate of ≤ 30%. Biochemical pregnancy rate was defined as a positive hCG (hCG > 6 IU/l) 17 days after oocyte retrieval. Clinical pregnancy was defined as the presence of gestational sac on ultrasound during the 6th week of gestation and live birth was defined as delivery of a neonate born ≥ 24 weeks of gestation. All outcomes are reported as per cycle initiated.

Statistical analysis

Since the goal of the study was to identify the set of covariates that best predicted low fertilization, we randomly divided the 663 treatment cycles into two halves by using a random number generator. The first half served as the training set (392 cycles) and was used to identify the most predictive set of covariates. The second half served as the validation set (391 cycles) where the results of the findings from the training dataset were corroborated. Descriptive statistics were calculated and compared for demographic and reproductive characteristics between these two datasets. A chi-square test or Fisher's exact test was used to test for differences across categories for discrete variables and the Kruskal-Wallis test for differences across categories for continuous variables.

Logistic regression with fixed effects was used to predict the risk of fertilization rate ≤ 30%. A stepwise variable selection process was used to identify predictors associated with low fertilization. Variables were allowed to enter the model when the univariable p value was < 0.05 and remained in the model if they remained associated with low fertilization rate at $p < 0.05$ when other variables entered the model. In order to avoid overrepresentation of cycles with few available oocytes for fertilization, we weighted the models by the total number of M2 oocytes retrieved. The female partner individual-level variables that were considered in this automated selection process were age (continuous, years), BMI (continuous, kg/m^2), ethnicity (discrete, non-Hispanic Caucasian vs. all others), initial infertility diagnosis (discrete, idiopathic infertility vs. all others), and antral follicle count classified into three discrete levels: diminished ovarian reserve count (< 6 follicles), normal antral follicle count (6–24 follicles, reference), and polycystic morphology (≥ 25 follicles) [14]. The male partner individual level variables considered were sperm concentration (continuous, million/ml), normal morphology (continuous, percent), and total progressive motility after swim-up processing (continuous, million/ml) from the most recent pretreatment semen analysis, as well as sperm concentration (continuous, million/ml), total motile count (continuous, million/ml), and postprocessing sperm progressive motility (continuous, percent) from the semen sample obtained on the day of egg retrieval. The cycle level variables considered were stimulation protocol (luteal phase GnRH agonist [0.25 mg per day], regular dose luteal leuprolide [0.5 mg per day], leuprolide flare, or GnRH antagonist); assisted hatching (binary), duration of infertility (continuous, months), day 3 FSH (IU/l), and day 3 estradiol (continuous, pg/ml); estradiol (continuous, pg/ml) at ovulation trigger day, day of hCG trigger (continuous), and number of ampules of IVF medications used (continuous, units).

The cutoff values for each significant predictor remaining in the model were identified through an iterative process where the logistic regression models were fitted that included all the significant predictors. In each iteration, predictors were individually dichotomized at different values, in increments of 5 units. The predictor value that maximized the significant odds ratio for low fertilization was retained. Next, each cycle was classified according to the number of dichotomized risk factors and estimates were obtained of the odds ratio for low fertilization for cycles with increasing number of risk factors relative to cycles without any risk factor.

The predictors and cutoffs from model fit from the validation set were incorporated with those from the training set. For all models, the receiver operating characteristic (ROC) curve statistics, the training and validation models' overall area under the curves (AUC) with their respective 95% confidence interval (CI), and the p value of the difference among the training and validation sets' AUC were analyzed. The individual odds ratios (ORs) for each predictor from their regression coefficient were predicted. All analyses were conducted using the Statistical Analysis System Software package SAS 9.4

(SAS Institute Inc., Cary, NC) and considered two-sided significance levels less than 0.05 as statistically significant.

Results

Sixty-six of the 663 cycles had a fertilization rate ≤ 30%, including 32 cycles with absent fertilization utilizing male partner's autologous sperm. The most common initial infertility diagnosis was idiopathic infertility (49%). Overall, the mean fertilization in the training and validation sets was 71%. Patient characteristics for the training and the validation sets are shown on Table 1. There were no differences between the training and validation sets with respect to baseline demographic and clinical characteristics, response to ovulatory induction (amount of gonadotropins used, day of hCG trigger, estradiol on day of hCG trigger, etc.), fertilization rate, and clinical treatment outcomes (clinical pregnancy and live birth rates). Semen parameters were comparable between the training and validation sets although sperm morphology was lower (but within the normal range) in the validation group compared to the training group (7.0% vs. 8.0%; p value = 0.02). There were no differences between pregnancy or ongoing pregnancy rates between the two groups, but there was a higher live birth rate in the training set per cycle initiated.

Stepwise regression retained only two significant predictors: postprocessing sperm concentration and postprocessing sperm progressive motility (Table 2). The cutoff value for postprocessing concentration was 40 million/ml and for postprocessing progressive motility was 50%. Overall, 150 of the couples in the training set and 154 in the validation set met had either postprocessing concentration or progressive sperm motility below these parameters. In the training set, the odds of low fertilization were 3.57-fold higher in cycles with a postprocessing concentration below 40 million/ml and 6.2-fold higher for cycles with a postprocessing progressive motility below 50% (Table 3). The results were similar, albeit reduced, in the validation set (Tables 2 and 3). Additionally, the change in concentration between the pre- and postwash samples were evaluated and were not related to low fertilization in the multivariate analysis model.

Each cycle was classified based on having postprocessing concentration or progressive sperm motility below the cutoff. Cycles with one or both adverse variables were compared relative to cycles without either predictor (Table 4, Supplemental Table 1). The presence of either low postprocessing concentration or low postprocessing progressive motility was associated with a 5-fold greater odds of fertilization failure in the training set (Table 4). Moreover, having low levels of both postprocessing concentration and progressive motility was related to a 22-fold greater odds of low fertilization in the training set. These associations were reduced in the validation set, where cycles with adverse values of both postprocessing semen parameters had a 13-fold greater odds of low fertilization (Table 4).

Discussion

In the absence of abnormal pretreatment semen parameters, low fertilization following conventional insemination during the first IVF attempt has been difficult to predict. In addition to limited fertilization, these cycles frequently result in limited embryo cohorts (number and quality) and may also result in diminished clinical outcomes. The present study investigated the impact of male, female, and IVF cycle characteristics on the rate of normal fertilization. We observed that fresh ejaculate sperm concentration and total progressive motility on the day of the oocyte retrieval were predictive of low fertilization in IVF conventional insemination cycles. To our knowledge, this is the first study to report specific factors that predict low fertilization during first attempt conventional insemination IVF cycles.

A comprehensive evaluation of risk factors for low or no fertilization in couples without identifiable indications for ICSI has not been reported previously. Studies investigating conventional insemination with low fertilization were primarily conducted more than a decade ago, did not establish semen parameter threshold for low or no fertilization, were comprised of relatively small numbers of (fewer than 150 patients), and primarily identified only male-related factors [6, 15]. In an early observational study, abnormal parameters for sperm viability, motility, and morphology were associated with low fertilization [1]. A retrospective cohort by Repping et al., it was reported that the decreased total motile sperm count on the day of the egg retrieval increased the likelihood of fertilization failure (zero 2PNs). However, the investigators of this study were unable to determine a specific cut off value to predict fertilization failure [16]. In patients who have had a history of fertilization failure with ICSI and were undergoing a subsequent cycle, higher numbers of oocytes retrieved, mature oocytes and higher estradiol at trigger were associated with improved fertilization during the subsequent attempt [17]. In contrast to prior studies, our study examined the largest population over a 10-year period of time to establish thresholds for semen parameters on the day of oocyte retrieval which identify patients with an increased risk of low fertilization that may be clinically actionable for providers.

It has been speculated that the inability to predict low fertilization during conventional insemination has contributed to the 51.5% increase in ICSI utilization for non-male infertility [18]. However, lower pregnancy rates in couples undergoing elective ICSI have also influenced the recommendation of several investigators not to implement the universal use of ICSI for all IVF patients [5, 19, 20]. It has been estimated that ICSI would need to be utilized in 33 cases to prevent

Table 1 Baseline characteristics of the participants ($N = 663$ women)

	Training set *N* (%) or median (IQR)	Validation set	*p* value
Characteristic	332 (45.5)	331 (45.3)	0.97
Demographics			
Age (years)	35.0 (32.9, 38.4)	35.5 (32.9, 38.0)	0.71
BMI (kg/m^2)	22.9 (21.0, 25.6)	23.0 (21.0, 26.2)	0.61
Caucasian race	245 (73.8)	263 (79.5)	0.09
Infertility diagnosis			0.82
Diminished ovarian reserve	36 (10.9)	34 (10.3)	
Ovulatory dysfunction	17 (5.1)	14 (4.3)	
Endometriosis	50 (15.1)	54 (16.4)	
Tubal factor	54 (16.3)	46 (14.0)	
Uterine factor	7 (2.1)	12 (3.7)	
Other causes	3 (0.9)	5 (1.5)	
Idiopathic	164 (49.6)	164 (49.9)	
Duration of infertility (months)	14 (12, 24)	15 (12, 24)	0.81
Day 3 FSH (IU/l)	7.0 (6.0, 8.6)	6.9 (5.8, 8.4)	0.18
Day 3 estradiol (pg/ml)	42 (31, 54)	40 (31, 54)	0.85
Antral follicle count	12 (9, 18)	12 (8, 18)	0.94
Anti-Müllerian hormone (ng/ml)	1.8 (1.0, 3.6)	1.8 (1.1, 3.8)	0.88
Cycle stimulation			
Stimulation protocol			0.99
Luteal phase GnRH agonist	242 (72.8)	242 (72.1)	
GnRH agonist flare	42 (12.7)	39 (11.8)	
GnRH antagonist	48 (14.5)	50 (15.1)	
Estradiol at trigger (pg/ml)	2069 (1509, 2523)	1960 (1426, 2620)	0.73
IVF cycle day of HCG trigger	12 (11, 13)	12 (11, 13)	0.39
Total IVF medications (IU)	2700 (1744, 3788)	2700 (1575, 3750)	0.63
Total oocytes retrieved	11 (7, 15)	10 (7, 14)	0.24
Mature oocytes retrieved	9 (6, 3)	9 (6, 13)	0.15
Number oocyte fertilized	6 (4, 9)	6 (3, 9)	0.04
Fertilization			
Fertilization (%)	71.8 (57.1, 83.3)	70.0 (53.3, 83.3)	0.49
Fertilization rate ≤ 30%	27 (8.1)	39 (11.8)	0.12
Embryos			
No. cleaved embryos	6 (4, 9)	5 (3, 9)	0.06
No. embryos transferred	2 (2, 2)	2 (1, 2)	0.92
Day of embryo transfer			0.94
Day 3	163 (49.1)	160 (48.3)	
Day 5	126 (40.0)	122 (36.9)	
No transfer performed	19 (5.7)	23 (7.0)	
Pretreatment semen analysis			
Initial sperm concentration (million/ml)	82.4 (52.0, 117.7)	84.1 (51.9, 122.8)	0.41
Initial sperm motility (%)	35.5 (27.0, 44.0)	33.0 (26.0, 41.0)	0.07
Initial normal morphology (%)	8.0 (6.0, 10.0)	7.0 (6.0, 10.0)	0.02
Postswim up total progressive motility	7.7 (4.5, 11.7)	7.5 (4.4, 11.2)	0.69
Day of retrieval semen analysis			
Postprocessing concentration (million/ml)	49.0 (27.0, 81.0)	45.0 (26.0, 75.0)	0.26
Postprocessing progressive motility (%)	67.0 (59.0 74.0	68.0 (59.0, 75.0)	0.29
Postprocessing motility (million/ml)	32.0 (17.0, 53.5)	30.0 (16.0, 49.0)	0.33
Clinical outcomes*			
Embryo transfer	312 (94.3)	308 (93.1)	0.52
Biochemical pregnancy rate	179 (53.9)	159 (48.0)	0.13
Clinical pregnancy	178 (53.6)	158 (47.7)	0.13
Live birth	154 (46.4)	125 (37.8)	0.02
Twin live birth (≥ 2 neonates)	33 (9.9)	30 (9.1)	0.70
Birth weight (g)	3107 (2721, 3570)	3025 (2608, 3425)	0.20

BMI body mass index, *GnRH* gonadotrophin-releasing hormone agonist, *IVF* in vitro fertilization, *FSH* follicle-stimulating hormone, E_2 estradiol, *hCG* human chorionic gonadotrophin

[a] Continuous variables are presented as median (interquartile range: 25th percentile, 75th percentile) while categorical variables are presented as number of women (percent)

[b] From Kruskal-Wallis test for continuous variable, and chi-squared test for discrete variables

[*] All outcomes are reported as per cycle initiated

Table 2 Odds ratio for low fertilization rate predictors

Characteristic	Low fertilization rate odds ratio (95% CI)	
	Training set ($N = 332$)	Validation set ($N = 331$)
Postprocessing sperm concentration (million/ml)	0.97 (0.96, 0.99)	0.98 (0.96, 0.99)
Postprocessing sperm progressive motility (%)	0.94 (0.91, 0.97)	0.96 (0.93, 0.98)
	Area under the curve (95% CI)	
	0.72 (0.67, 0.83)	0.66 (0.56, 0.76)

Data is presented as the effect of 1 unit of increase in the odds ratio in relation to having a low fertilization (≤ 30% fertilization rate). Estimates were calculated using stepwise selection of the following predictors: age (years), BMI (kg/m^2), ethnicity, infertility diagnosis, antral follicle count, IVF cycle type, duration of infertility (months), FSH levels at day 3 (pg/ml), estradiol levels at day 3 and at trigger (pg/ml), day of HCG trigger, units of IVF medication utilized for induction (IU), initial immature round cells (mil/ml), initial concentration (mil/ml), initial normal morphology (%), initial progressive motility (%), postprocessing progressive motility (%). Model was weighted by the total number mature (M2) oocytes retrieved

one occurrence of fertilization failure [5]. The literature on the safety of ICSI warrants additional investigation with conflicting results with respect to adverse long-term outcomes [21–24]. The impact of the widespread use of ICSI on healthcare system expenditures or resulting offspring has yet to be fully appreciated. However, following one fresh IVF cycle with failed fertilization, the estimated recurrence rates for future cycles is 30% [25, 26]. The findings of our study may allow clinicians to better identify IVF cycles “at risk” for suboptimal fertilization with conventional insemination and provide guidance for recommending ICSI on a more selective basis as a means to potentially improve fertilization results.

A primary strength of this study is the inclusion of female demographic, fertility, and stimulation factors in addition to male semen parameters in our analyses. By using half the patients in the training set, the validation set was used to account for confounding variables (such as few numbers of oocytes and fertility diagnosis) on the outcome. The univariable analysis provides a comprehensive model to identify those with a positive score (concentration below 40 million/ml, total progressive motility less than 50% factor, or both). The probability of fertilization failure was highest when both the concentration and total progressive motility were below the cutoff, Supplemental Table 2. In models presented herein, having either low concentration and/or total progressive motility on the day of retrieval has a positive predictive value of 87.3% and a negative predictive value of 4.4%. The overall sensitivity was 70.4%, with a specificity of 57.0%.

There are several limitations of this study. Due to the retrospective nature, there may have been factors that influenced the clinical decision-making that were not reflected in the data collected. An example includes the 100 patients (excluded from our study) with normal pretreatment and day of egg retrieval semen parameters who utilized ICSI without clear indications. Approximately half of those subjects had idiopathic infertility, which may reflect increased utilization in those couples whose infertility remains unexplained. Additionally, the fertilization was based on the number of mature oocytes retrieved. In couples undergoing ICSI, cumulus cells are stripped from the oocytes to allow for definitive assessment of oocyte maturity. In conventional insemination, assessment

Table 3 Predictors of low fertilization

Criteria	Low fertilization rate odds ratio (95% CI)	
	Training set ($N = 332$)	Validation set ($N = 331$)
Postprocessing sperm concentration ≤ 40 million/ml vs. > 40 million/ml	3.57 (1.41, 9.01)	3.34 (1.58, 7.06)
Postprocessing sperm progressive motility ≤ 50% vs. > 50%	6.20 (2.29, 16.75)	2.90 (1.08, 7.83)
	Area under the curve (95% CI)	
	0.67 (0.56, 0.77)	0.61 (0.53, 0.70)

Data are presented as the effect of the exposure discrete category compared to the reference non-exposure category odds ratio in relation to having a fertilization failure (≤ 30% fertilization rate). Estimates were calculated the presence/absence of predictors: postprocessing semen concentration ≤ 40 million/ml and postprocessing semen progressive motility ≤ 50%. Model was weighted by the total number mature oocytes retrieved

Table 4 Predictive model for low fertilization rate

Criteria	Dataset	
	Training ($N = 332$)	Validation ($N = 331$)
Positive score (one or two positive criteria) vs. none		
Fertilization rate ≤ 30% odds ratio (95% CI)	5.08 (1.83, 14.12)	2.90 (1.40, 6.18)
Area under the curve (95% CI)	0.64 (0.55, 0.73)	0.60 (0.52, 0.68)
Only one positive criteria vs. none		
Fertilization rate ≤ 30% odds ratio (95% CI)	3.88 (1.34, 11.24)	2.27 (1.04, 4.97
Area under the curve (95% CI)	0.67 (0.56, 0.78)	0.62 (0.53, 0.71)
Two positive criteria vs. none		
Fertilization rate ≤ 30% odds ratio (95% CI)	22.07 (5.41, 90.03)	13.44 (4.01, 45.06)
Area under the curve (95% CI)	0.67 (0.56, 0.78)	0.62 (0.53, 0.71)

Data is presented as odds ratio of the effect of the exposure discrete category comparing having a positive score against in relation to having a low fertilization (≤ 30% fertilization rate). Model was weighted by the total number mature oocytes retrieved

of oocyte maturity is not as accurate as ICSI, since cumulus stripping is not performed and may lead to an overestimation of oocyte maturity. The assumption is that these oocytes were mature at time of insemination and thus negatively impact fertilization rate. An additional limitation to consider is that history of prior paternity was inconsistently and incompletely captured in our electronic medical record system and could not be included as a variable. Lastly, the patients that had low fertilization often required additional cycles of IVF to obtain pregnancy, which was not reviewed here; however, the result of the first IVF cycle remains a pivotal point for clinical decisions of subsequent cycles. While many studies have shown improved outcomes utilizing ICSI after previous low fertilization, others have not corroborated those findings [4, 27, 28]. Furthermore, a study by Tomas et al. showed lower pregnancy rates in those with prior low fertilization when compared to couples with male factor infertility utilizing ICSI during subsequent IVF cycles [29], raising the issue of whether ICSI would have prevented the low fertilization during prior cycles. Unfortunately, it has not been demonstrated if preemptive ICSI performed due to the thresholds for semen parameters we report would result in better fertilization rate and/or improved live birth rates in all couple with a variety of infertility diagnoses. This highlights the complex interplay of multiple factors impacting fertilization that requires further investigation.

In conclusion, specific semen parameters on the day of oocyte retrieval are associated with low and absent fertilization during conventional IVF cycles even with normal pretreatment semen parameters. The findings of this study provide further insight into possible predictors of suboptimal fertilization outcomes. Unanticipated low semen concentration and/or progressive motility on the day of egg retrieval in cycles with planned conventional insemination may warrant a discussion of ICSI with the goal to prevent low fertilization. If validated by prospective investigation, these data may have the potential to optimize ART results by identifying those at risk for low fertilization.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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