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Sperm morphology: What implications on the assisted reproductive outcomes?

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Abstract

Objective: To evaluate the impact of sperm morphology (SM) on laboratory and pregnancy outcomes in conventional intracytoplasmic sperm injection (c-ICSI) cycles, using the egg donation model to minimize female confounding variables.

Materials and methods: We retrospectively collected data of oocyte donation cycles from October 2016 to February 2020. Median seminal parameters, total (1-2-3PN) fertilization rate (FR), 2PN FR, cleavage rate (CR), implantation rate (IR), pregnancy rate (PR), miscarriage rate (MR), and live birth rate (LBR) were collected. The study population was divided into three groups: Group 1 with SM < 4%, Group 2 with SM between 4% and 6%, and Group 3 with SM > 6%.

Results: Of 741 fresh ICSI cycles and 4507 warmed oocytes were included. Male age was 46.0 (31.0-72.0) years, and recipients' age was 44.0 (29.0-54.0) years. Normal SM was 5.0% (1.0%-15.0%). Male age was negatively correlated with normal SM ($P = .002$; Rho −0.113). Oocyte survival rate was 83.3% (16.7%-100.0%). Total FR was 75.0% (11.1%-100.0%), 2PN FR was 66.7% (11.1%-100.0%) %, and CR was 100% (0.0%-100%). Comparing samples with SM > 6% and those with SM < 4%, 2PN FR was significantly higher in the first group ($P = .04$). No significant associations were found among groups in terms of CR. IR was 27.7%, resulting significantly higher when normal SM was > 6% ($P < .01$). Clinical PR was 36.0%, MR was 23.9%, and LBR was 25.9%. PR and LBR were significantly higher in samples with normal SM > 6%, compared to other groups ($P = .02$ and $P < .01$, respectively).

Conclusions: Although c-ICSI technique allows the embryologist to select the best quality spermatozoa, male factor plays a key role in achieving successful assisted reproductive outcomes. Normal SM has been shown to have implications not only for laboratory outcomes, in terms of fertilization, but also for clinical findings, as regards implantation, pregnancy, and live birth.

KEYWORDS

assisted reproduction, morphology, oocyte donation, semen analysis, spermatozoa

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1 | INTRODUCTION

Infertility may be due to male, female, or idiopathic factors. Male infertility affects about 7% of men,[1] resulting in most cases in alterations of sperm concentration, motility, and morphology. Other factors, such as seminal markers of epididymal, prostatic, and seminal vesicle function, could play a minor role in causing infertility.[2]

Morphological changes that occur during spermiogenesis in humans are not exactly homogeneous, leading to spermatozoa with various morphological abnormalities. Therefore, as happened 30 years ago, several authors have searched for describing morphologically normal spermatozoa.[3]

Human spermatozoa have a peculiar arrangement characterized by an oval-shaped head (3-5 μm length and 2-3 μm width), a midpiece (7-8 μm), and a tail (45 μm). A dense nucleus, covered by acrosome and plasma membrane, constitutes 40%-70% of sperm head. In particular, the acrosome includes a mixture of proteins and hydrolytic enzymes, crucial for sperm binding to the egg zona pellucida, known as acrosome reaction. From the nucleus base, core microtubules develop from the distal centriole to generate outer dense filaments in the midpiece area, drawn by mitochondria. Therefore, the junction of midpiece nuclear pole to the base of sperm head shapes the neck. The conclusive phase of spermiogenesis results in the creation of sperm tail, finally originating morphologically normal and mature spermatozoa.[4,5] Thus, spermatogenesis hitches could cause sperm production with abnormal forms.[6]

Sperm morphology (SM) proved to be the more suitable predictor of fertilization potential, rather than other seminal parameters, such as motility and concentration. Given the central role of morphology as a viable tool in guiding the decision-making of clinical practice, the WHO's definition of teratozoospermia has changed over time, due to the decline in semen quality reported in recent years. The reference range for normal SM moved from 30% in 1992 to 14% in 1999 and finally to 4% in 2010.[7] Although some laboratories do not consider SM quite important,[8] several studies have demonstrated that poor sperm quality and SM are associated with higher incidence of aneuploid embryos, suggesting that the meiotic events occurring during fertilization could impact on the distribution of chromosomes.[9,10]

Moreover, comparing couples which men have normal SM < 4% and those with SM ≥ 4% in in vitro fertilization (IVF) cycles, a significant reduction in fertilization rates was found in the first group, despite the lack of significant differences on clinical and neonatal outcomes.[11]

Thus, the aim of this study was to evaluate the potential impact of SM on laboratory and pregnancy outcomes in conventional intracytoplasmic sperm injection (c-ICSI) cycles, using the oocyte donation model to minimize all possible female confounding variables.

2 | MATERIALS AND METHODS

2.1 | Study population

We retrospectively collected data of all oocyte donation cycles performed from October 2016 to February 2020 at our Assisted Reproductive Technologies (ARTs) Center, University of Florence (Italy). All the couples gave their written informed consent to have records included into the study. The study was conducted in accordance with the Declaration of Helsinki and after obtaining the Institutional Review Board (IRB) approval (CEAVC, reference no. 10189 and no. 14804). All female recipients suffered from severe infertility factor, including diminished ovarian reserve, poor ovarian response, repeated unsuccessful homologous IVF cycles, or premature ovarian failure.

All anonymous egg donors, recruited from foreign banks, were 18-25 years old and fulfilled the standard screening criteria as outlined by the American Society of Reproductive Medicine (ASRM). All female donors had a good health, regular monthly periods, and no gynecological complaints.

The study population was divided into three groups, according to normal SM proportion: Group 1 included men with SM < 4%, Group 2 comprised men with SM between 4% and 6%, and Group 3 included men with SM > 6%.

2.2 | Recruitment criteria

Criteria for inclusion were as follows: couple infertility for one year onwards, severe female infertility factor that requires the egg donation program, cycles resulted in embryo transfer (ET), and anterograde ejaculation. Exclusion criteria were as follows: azoospermia and thawing cycles with overall oocyte survival rate ≤ 15.0%.

2.3 | Baseline assessment

All couples performed a complete medical work-up, involving a gynaecological evaluation and uro-andrological investigations. Demographic characteristics, including male and female age, were reported.

On males, urine and semen culture were required to exclude the presence of urinary or seminal tract infections. Examinations for HIV 1/2, HCVab, HbsAg, HbsAb, HBcAb, Treponema Pallidum Hemagglutination and Venereal Disease Research Laboratories (TPHA-VDRL), Ab anti-Chlamydia Trachomatis, and Ab anticytomegalovirus (CMV) were performed in both partners.

Semen collection was performed by fresh on the same day of oocyte thawing. Samples were evaluated in accordance with the 2010 World Health Organization (WHO) standards (5th edition)[12] by two operators with great deal of expertise in this field. Each sample was obtained by masturbation after 3-5 days of sexual abstinence. We collected seminal parameters as follows: volume, pH, sperm concentration (million/ml), total sperm count (million/ejaculate), viability, progressive motility (PR), non-progressive motility (NP), immobility (IM), total motility, and morphology.

2.4 | Laboratory

For the morphology assessment, each sample was stained using the Diff-Quik (DQ) staining technique, following the WHO manual

guidelines.[12] We smeared 10uL of semen on a slide, which was fixed by immersion in triarylmethane fixative for 15 seconds after complete air drying. The smears were then consecutively stained by solution 1 (10 seconds), then air-dried, and stained by solution 2 (5 seconds). Finally, the slides were washed in running tap water to remove the excess stain (10 to 15 times). The stained slides were read at 1000 magnification with oil immersion within 2 hours of their preparation. We examined 200 spermatozoa, in order to achieve an acceptably low sampling error, in two independent replicates. Tygerberg strict criteria were used for the evaluation of the percentage of morphologically normal and abnormal spermatozoa, by following the WHO guidelines.[12] Replicate values were compared to check whether they are acceptably close (according to the method for the comparison of replicate percentages reported in WHO). The assessment was repeated if the difference was not acceptable.

Our laboratory participates in two external quality assurance scheme—the UK National External Quality Assurance Scheme (UK NEQAS) and the external quality control (EQC) of Tuscany Region (Italy)—to periodically verify that the assessments comply with these criteria.

2.5 | Main outcome measures

Median number of thawed and survived oocytes was collected. All donor oocytes that endured the post-thawing procedure went to ICSI. Consequently, overall oocyte survival rate (% no. survived/no. thawed) was reported. Oocytes were examined under a microscope 18-20 hours after insemination to verify whether fertilization has occurred or not. Median number of total fertilized oocytes (1-2-3 pronuclear—PN), normal fertilized oocytes (2PN), total embryos obtained, cryopreserved embryos, arrested embryos, and embryos transferred was collected. Accordingly, the main ICSI outcome measures included total and normal fertilization rates (FR), calculated as the total number of fertilized oocytes and 2PN fertilized oocytes by the number of injected oocytes, respectively. Cleavage rate (CR) was calculated as the number of embryos obtained by the number of normal fertilized oocytes. The day of ET into the uterine cavity (48 to 72 or 120 hours after ICSI) was reported.

By calculating median number of implanted embryos in intrauterine and extrauterine cavity, the implantation rate per transfer was obtained. Implantation rate (IR) was defined as the total sacs number on the total number of embryos transferred. Therefore, the main pregnancy outcomes per cycle or transfer were collected: clinical pregnancy rate (PR), miscarriage rate (MR), and live birth rate (LBR).

After 14 days from ET, human chorionic gonadotropin (HCG) dosage was measured. Clinical pregnancy was defined by HCG levels above 50 mU/L and documented by transvaginal ultrasound visualization of intrauterine gestational sac with heartbeat at around 5-6 weeks of gestation. Pregnancy loss before 20 weeks of gestation and biochemical pregnancies were considered as miscarriages. LBR was defined as the percentage of all cycles that led to live birth, by evaluating ET performed until August 2019. Median number of single, twin, and trigeminal deliveries was collected.

2.6 | Statistics

The groups were compared using odds ratio (OR) and a 95% confidence interval (CI). Differences in frequencies between groups were tested with the chi-square method. The Fisher exact test was used as appropriate. Continuous variables are presented as median (range). Differences between groups were assessed by the Student independent t test or the Mann-Whitney U test based on their normal or not-normal distribution, respectively. The Kruskal-Wallis test was used for testing the baseline participants' parameters. Analyzing the distribution of sample proportion with normal SM, the cutoff of 4% corresponds to the 15th centile of the distribution. Thus, we classified the study sample into three groups: the tails of the distribution (left and right, corresponding to the 15th and 85th centiles, respectively) and the rest central area (which corresponds to the 4%-6% range of normal sperm morphology). This division, in addition to having a statistical decision-making basis, is also suggested by observing the histogram of the distribution in which the 15th and 85th centiles reflect two natural breaks. Spearman's Rho test was used to measure the strength of associations between male age and semen parameters and to evaluate the correlation between semen parameters and ICSI outcomes. The independent factors with a possible influence on the assessed outcomes were also evaluated by a logistic regression analysis, including normal sperm morphology, male and recipients' age, and the number of embryo transferred as covariates/adjustment factors/possible confounders. A p-value < 0.05 was considered significant. Statistical analysis was performed with the use of the Statistical Package for the Social Sciences (SPSS, version 26.0, IBM).

3 | RESULTS

This observational single-center retrospective study included 741 ICSI cycles and 4507 warmed oocytes. Finally, 1332 patients (666 couples) were enrolled for data analysis. Median male age was 46.0 (31.0-72.0) years, and recipients' age was 44.0 (29.0-54.0) years. Semen parameters of the three groups are listed in Table 1. Group 1 (SM < 4%) included 91 cycles (12.3%), Group 2 (SM 4%-6%) comprised 525 cycles (70.8%), and Group 3 (SM > 6%) involved 125 cycles (16.9%). Median normal SM was 5.0 (1.0-15.0) %. SM alteration goes along with the impairment of all seminal parameters, including volume, pH, sperm concentration/ml, sperm count/ejaculate, viability, motility, and sperm concentration with progressive motility (Table 1). As indicated, men belonging to the Group 3 (SM > 6%) showed seminal characteristics significantly higher ($P < .05$).

As shown in Figure 1, male age was negatively correlated with normal SM ($P = .002$; Rho Spearman −0.113). All seminal parameters

TABLE 1 Baseline characteristics of the study population, according to normal sperm morphology (SM) percentage

	Group 1 (SM < 4%)	Group 2 (SM 4%-6%)	Group 3 (SM > 6%)	Total	*P*-value
Male age, years	47.0 (33.0-60.0)	46.0 (31.0-61.0)	44.0 (31.0-59.0)	46.0 (31.0-61.0)	.01*
Recipient age, years	44.0 (32.0-52.0)	44.0 (29.0-54.0)	44.0 (30.0-52.0)	44.0 (29.0-54.0)	.84
Semen volume, ml	2.9 (0.2-7.5)	2.8 (0.3-8.5)	2.7 (0.5-8.1)	2.8 (2.8- 8.5)	.48
Semen pH	7.8 (2.3-8.5)	7.6 (6.6-8.0)	7.6 (2.6-8.0)	7.6 (2.3-8.5)	.04
Sperm concentration, million/ ml	5.3 (0.1-134.0)	38.0 (0.4-205.0)	80.0 (2.7-200.0)	35.0 (0.1-205.0)	<.01*
Total sperm count, million/ ejaculate	14.9 (0.1-536.0)	92.0 (0.9-616.0)	186.0 (7.6 1440.0)	90.0 (0.1-1440.0)	<.01*
Viability, %	60.0 (2.0-90.0)	72.0 (18.0-92.0)	81.0 (57.0-92.0)	72.0 (2.0-92.0)	<.01*
Progressive motility (PR), %	30.0 (0.0-75.0)	50.0 (0.0-85.0)	62.0 (30.0-85.0)	45.0 (0.0-85.0)	<.01*
Non-progressive motility (NP), %	10.0 (0.0-50.0)	10.0 (0.0-60.0)	5.0 (0.0-20.0)	10.0 (0.0-60.0)	<.01*
Immobility, %	60.0 (20.0-100.0)	40.0 (10.0-98.0)	30.0 (15.0-60.0)	45.0 (10.0-100.0)	<.01*
Total Motility (PR + NP), %	40.0 (0.0-80.0)	60.0 (2.0-90.0)	70.0 (40.0-85.0)	55.0 (0.0-90.0)	<.01*
Sperm concentration with progressive motility (PR), million/ml	2.9 (0.1-402.0)	39.6 (0.1-493.0)	113.1 (3.0-1080.0)	38.5 (0.1-1080.0)	<.01*
Normal sperm morphology (SM), %	3.0 (1.0-3.0)	5.0 (4.0-6.0)	7.0 (7.0-15.0)	5.0 (1.0-15.0)	<.01*

Note: All variables are expressed in median (range). A *P*-value < .05 was considered significant (*).

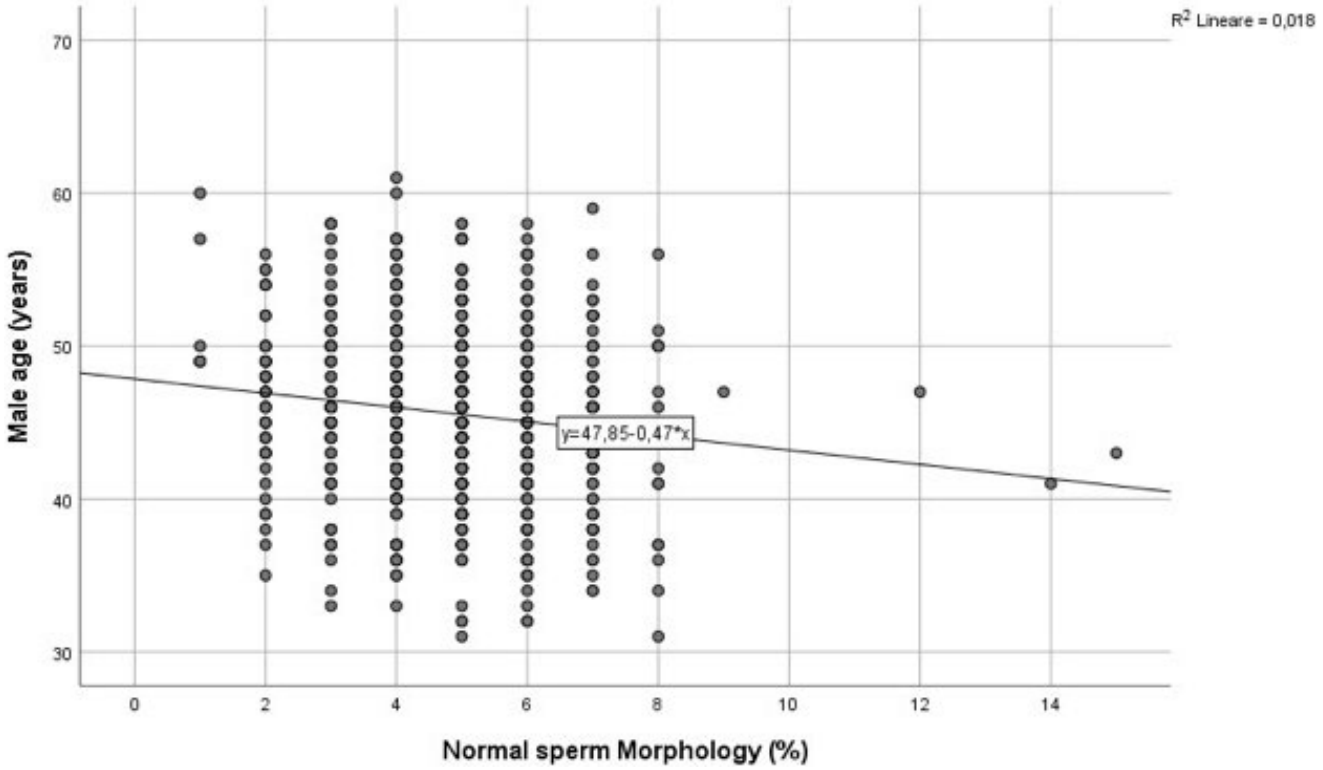

FIGURE 1 Correlation between male age and normal sperm morphology ($R^2 = 0.018$)

worsen with increasing male age: total sperm count, viability, and progressive motility (PR) significantly decreased according to advanced paternal age (Table 2).

3671 oocytes were survived and injected with fresh ejaculated spermatozoa. Median oocyte survival rate was 83.3 (16.7-100.0) %. As depicted in Table 3, median total FR was 75.0

TABLE 2 Correlation between male age and semen parameters

	P-value	Correlation coefficient
Normal sperm morphology (SM), %	.002*	−0.113
Sperm concentration, million/ ml	.479	−0.026
Total sperm count, million/ejaculate	.034*	−0.078
Viability, %	.000*	−0.165
Progressive motility (PR), %	.000*	−0.155

Note: Spearman's Rho test was used to measure the strength of associations between two variables. A *P*-value < .05 was considered significant (*).

(11.1-100.0) %, 2PN FR was 66.7 (11.1-100.0) %, and CR was 100 (0.0-100) %.

As depicted in Figure 2, in comparing samples with SM > 6% (Group 3) and those with SM < 4% (Group 1), significantly higher 2PN fertilization rates were detected in the first group ($P = .04$). No significant associations were found among groups in terms of CR.

Sperm concentration/ml and total sperm count/ejaculated were positively correlated with total FR ($P = .05$ and $P = .02$, respectively) and with 2PN FR ($P < .01$ and $P < .01$, respectively). Sperm viability was not associated with enhanced laboratory outcomes ($P > .05$). CR is not associated with sperm concentration/ml, total sperm count/ejaculate, and viability ($P = .33$, $P = .94$ and $P = .34$, respectively).

A median of 3.0 (1.0-8.0) embryos per cycle was obtained, and 2.0 (1.0-3.0) embryos were transferred. Overall, 2357 embryos were obtained. From 1622 usable, 1161 embryos were transferred. Median IR was 27.7%, resulting significantly higher when normal SM was > 6% ($P < .01$) (Table 4). Median clinical PR was 36.0%, MR was 23.9%, and LBR was 25.9%.

PR and LBR were significantly higher in samples with normal SM > 6%, compared to other groups ($P = .02$ and $P < .01$, respectively). There were no significant differences in ET distribution on day 3 and day 5 between the three groups (Table 5). Total LBR did not differ significantly between ET on day 3 and day 5 (29.6% vs. 29.2%, respectively; $P = .60$). Comparing the outcomes by embryos transferred = 1 and > 1, LBR was significantly higher in the second group (17.5% vs. 31.2%, respectively; $P < .01$).

4 | DISCUSSION

The main findings of this study claim that normal SM impacts on the assisted reproductive outcomes. Indeed, 2PN fertilization, implantation, pregnancy, and live birth rates are significantly higher when normal SM is > 6%.

Previous authors investigated the potential influence of SM on the ARTs outcomes. Chen et al found that normal SM affects FR, but not clinical and neonatal outcomes, in c-IVF homologous cycles. Specifically, normal SM < 4% was significantly related to higher rates of total fertilization failure, while clinical pregnancy, implantation, and miscarriage rates did not differ significantly compared to patients with normal SM ≥ 4%.[11] Likewise, lower fertilization and higher total fertilization failure rates in c-IVF cycles were detected in men with isolated teratozoospermia, as compared to those with normal semen profile.[13]

Moreover, also sperm concentration, count, and viability could be positively related to enhanced ICSI outcomes.[14] In our case series, sperm concentration/ml and total sperm count/ejaculate are confirmed to be positively associated with ICSI outcomes (total and 2PN FR), but not with CR, whereas sperm viability does not have a positive influence on laboratory outcomes.

It is widely known that embryo development might be influenced by the quality of spermatozoa used for fertilization.[15,16] Coban et al focused on the incidence of embryo aneuploidy and abnormal SM in oocyte donations. As main conclusion, total aneuploidy rate and its derivates (trisomy or monosomy) were considerably related to SM, suggesting a direct relationship between semen quality and embryo abnormalities in pre-implantation stage.[10]

As shown by our results, the assessment of SM could be considered a feasible biomarker of sperm fertilizing capacity, regardless of motility and concentration. In fact, normal (2PN) fertilization rates are significantly higher in samples with SM > 6%. To strengthen our data, a retrospective analysis of IVF cycles found FR considerably

TABLE 3 Intracytoplasmic sperm injection (ICSI) outcomes, according to normal sperm morphology (SM) percentage

	Group 1 (SM < 4%)	Group 2 (SM 4%-6%)	Group 3 (SM > 6%)	Total	*P*-value 1 vs. 2	*P*-value 2 vs. 3	*P*-value 1 vs. 3
Oocyte survival rate, %	83.3 (16.7-100.0)	83.3 (16.7-100.0)	83.3 (33.3-100.0)	83.3 (16.7-100.0)	.57	.72	.49
Total fertilization rate (FR), %	75.0 (16.7-100.0)	80.0 (11.1-100.0)	80.0 (33.3-100.0)	75.0 (11.1-100.0)	.12	.59	.11
2PN fertilization rate (FR), %	66.6 (16.7-100.0)	66.6 (11.1-100.0)	66.6 (20.0-100.0)	66.7 (11.1-100.0)	.09	.37	.04*
Cleavage rate (CR), %	100.0 (0.0-100)	100.0 (0.0-100)	100.0 (33.3-100)	100.0 (0.0-100)	.97	.43	.51

Note: All variables are expressed in median (range). A *P*-value < .05 was considered significant (*).

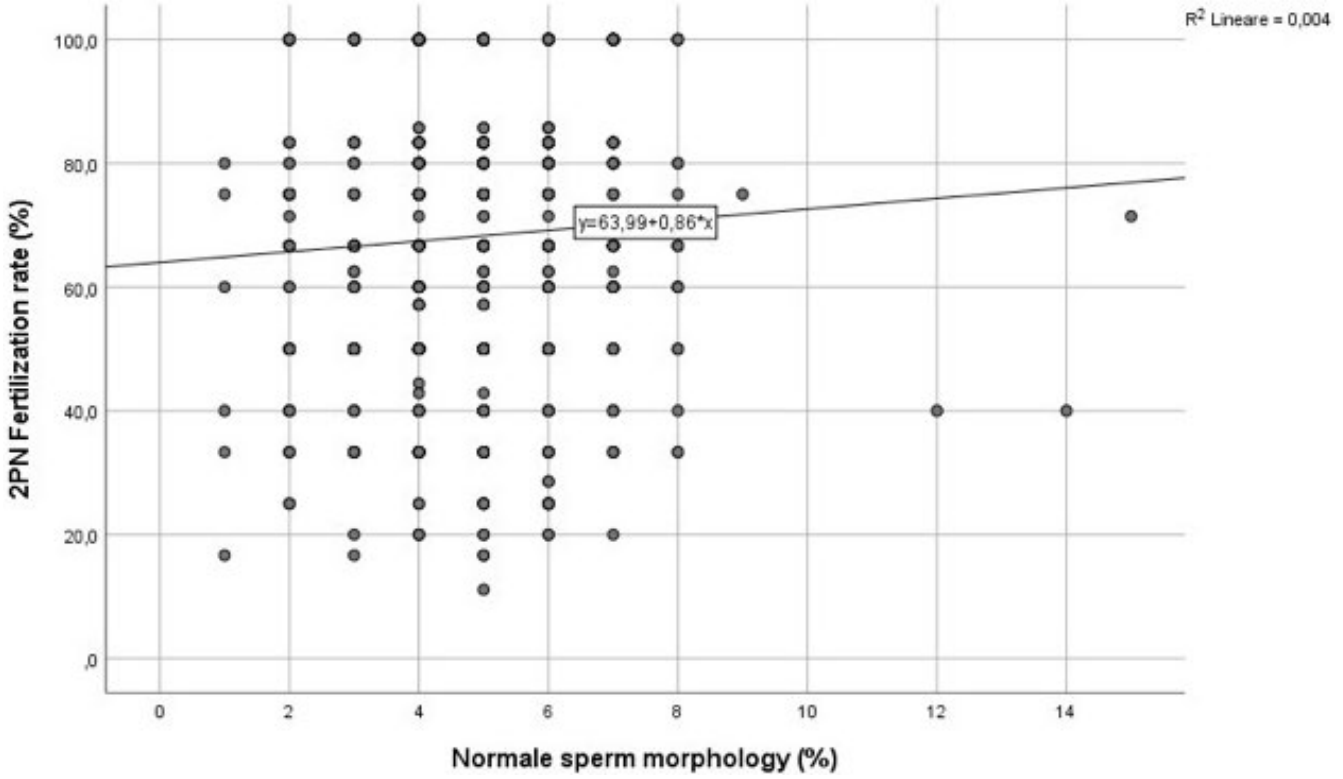

FIGURE 2 Correlation between 2 pronuclear (PN) fertilization rate and normal sperm morphology ($R^2 = 0.004$)

TABLE 4 Pregnancy outcomes, according to normal sperm morphology (SM) percentage

	Group 1 (SM < 4%)	Group 2 (SM 4%-6%)	Group 3 (SM > 6%)	Total	*P*-value 1 vs. 2	*P*-value 2 vs. 3	*P*-value 1 vs. 3
Pregnancy rate (PR), %	49/125 (39.2)	176/525 (33.5)	42/91 (46.1)	267/741 (36.0)	.25	.02*	.33
Implantation rate (IR), %	56/180 (31.1)	213/839 (25.3)	53/144 (36.8)	322/1163 (27.7)	.11	<.01*	.29
Miscarriage rate (MR), (%)	10/49 (20.4)	44/176 (25.0)	10/42 (23.8)	64/267 (23.9)	.07	.60	.09
Live birth rate (LBR), %	24/96 (25.0)	107/446 (23.9)	31/83 (37.3)	162/625 (25.9)	.04*	<.01*	.53
Singleton birth	25/31 (80.6)	78/100 (78.0)	22/31 (71.0)	125/162 (77.2)	1.00	.47	.55
Multiple birth	6/31 (19.4)	22/100 (22.0)	9/31 (29.0)	37/162 (22.8)			

Note: Live birth rate (LBR) was calculated considering cycles with embryo transfer (ET) until August 2019 (n = 625). A *P*-value < .05 was considered significant (*).

reduced in patients with normal SM < 4%, as well as lower implantation and ongoing pregnancy rates.[17]

We exclusively included in our study donor egg programs, to standardize the maternal component and to remove all possible confounding factors related to oocyte quality. Oocytes are derived from young healthy women who have met multiple strict criteria to qualify as donors. Thereby, we were able to give more emphasis to paternal aspects.

Furthermore, other elements could affect the main findings with regard to pregnancy outcomes. The advanced recipients' age and its derivates, such as the endometrial factor, could compromise successful implantation or, even more, pregnancy, delivery, or live birth.[18,19]

Nevertheless, as detected in our study, normal SM is able to affect successful pregnancy outcomes: Implantation, clinical pregnancy, and live birth rates resulted higher when normal SM was > 6%, irrespective of recipient's factors. To validate our data and avoid all possible bias, median female age was comparable among groups.

We are aware that ICSI is used to bypass the severe shortfalls related to potential seminal anomalies. However, low-quality spermatozoa proved to determine a negative influence on pregnancy outcomes even with ICSI, causing decreased blastocyst formation, increased chromosome aneuploidy, and decreased IR, PR, and LBR.[20] On the contrary, other authors did not find significant correlations between subnormal male sperm parameters and IVF outcomes, in a frozen oocyte donor model.[21]

TABLE 5 Live birth rate (LBR), according to embryo transfer (ET) day

	Group 1 (SM < 4%)	Group 2 (SM 4%-6%)	Group 3 (SM > 6%)	Total	*P*-value 1 vs. 2	*P*-value 2 vs. 3	*P*-value 1 vs. 3
ET Day 3, %	86/96 (89.6)	413/446 (92.6)	78/83 (94.0)	577/625 (92.3)	.30	.81	.41
ET Day 5, %	10/96 (10.4)	33/446 (7.4)	5/83 (6.0)	48/625 (7.7)			
ET with embryos transferred = 1, %	41/96 (42.7)	163/446 (36.5)	36/83 (43.4)	240/625 (38.4)	.29	.26	1.00
ET with embryos transferred > 1, %	55/96 (57.3)	283/446 (63.5)	47/83 (56.6)	385/625 (61.6)			
LBR—ET Day 3, %	22/86 (25.5)	99/413 (23.9)	27/78 (34.6)	148/577 (25.6)	.07	.03*	1.00
LBR—ET Day 5, %	2/10 (20.0)	8/33 (24.2)	4/5 (80.0)	14/48 (29.2)	1.00	.02*	.08
LBR—ET with embryo transferred = 1, %	6/41 (14.6)	28/163 (17.1)	9/36 (25.0)	42/240 (17.5)	.48	.21	.59
LBR—ET with embryo transferred > 1, %	18/55 (32.7)	79/283 (27.9)	22/47 (46.8)	120/358 (31.2)	.03*	<.01*	.69

Note: Live birth rate (LBR) was calculated considering cycles with embryo transfer (ET) until August 2019 (n = 625). A *P*-value < .05 was considered significant (*).

Moreover, morphology defects in the spermatozoa seem to be positively correlated with fragmented DNA both in whole semen and single retrieved cells. Indeed, DNA breaks and oxidative stress induced by impaired chromatin compaction could be the major reasons of poor semen morphology.[22,23]

However, although in c-ICSI cycles the embryologists aim to select an ideal spermatozoa at 400x-power magnification, relying on normal morphology, the percentage of spermatozoa with high DNA fragmentation could be greater than 55%, even in the amount of morphologically normal ones.[24,25] This could be one reason why, in our series, worst sperm morphology is related to lower fertilization rates.

Previous researchers have demonstrated that the intracytoplasmic injection of aneuploid or DNA damaged spermatozoa could undermine the embryo development.[26] Moreover, nuclear chromatin decondensation damages of spermatozoa could lead to decreased fertility potential, poor embryo quality, and pregnancy loss.[27,28]

Although some studies found that sperm DNA damage has minimal effects on fertilization,[29,30] we propose that male contribution to oocyte activation is a critical point of the successful outcomes. Indeed, the sperm plasma membrane and acrosome region may be regulated by the sperm-cumulus oophorus complex interface, leading to egg activation and improving fertilization rates.[31-33]

As a consequence, we stress the importance of the sperm quality, regardless of the ARTs technique. The rapid spread of ICSI put aside the clinician. Indeed, the embryologist was asked to select a single spermatozoon for injection, bypassing the usual process of natural selection which occurs during natural conception. On the contrary, our study goes against the common knowledge that ICSI may provide greater chances of fertilization with abnormal spermatozoa. On these bases, obtaining samples with higher percentages of normal SM, as well as concentration and count, becomes crucial for optimizing the outcomes. In this setting, the figure of uro-andrologist becomes essential for treating the male factor and improving semen quality before ICSI. However, more likely, this understanding could guide the clinician in counseling better the infertile couple who start an assisted reproduction program.

To the best of our knowledge, this is one of the larger studies that focus on the impact of SM on the ARTs outcomes, using the oocyte donation model. Since a potential correlation between abnormal SM and increased DNA fragmentation might exist,[32] the lack of data about sperm DNA fragmentation could represent a possible limitation of the study. Moreover, the advanced male age in the cohort could compromise the generalizability of the main study findings.

5 | CONCLUSION

Although c-ICSI technique allows the embryologist to select the best quality spermatozoa, male factor plays a key role in achieving successful assisted reproductive outcomes. Normal SM has been shown to have implications not only for laboratory outcomes, in terms of fertilization, but also for clinical findings, as regards implantation, pregnancy, and live birth.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

GC, RP, RF, and ME.C. conceptualized and designed, drafted the article, and acquired the data. CG, GC, EM, and PF acquired the data and analyzed and interpreted the data. AC, AM, MC, AN, and ME.C. critically revised the article. All of the co-authors interpreted the data and participated in finalizing the article. All of the co-authors approved the final version of the article.

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