ANDROLOGY: COHORT STUDY

Sperm deoxyribonucleic acid fragmentation (by terminal deoxynucleotidyl transferase biotin dUTP nick end labeling assay) does not impair reproductive success measured as cumulative live birth rates per donor metaphase II oocyte used

Irene Hervás, M.Sc.,[a] Alberto Pacheco, Ph.D.,[b,c] Maria Gil Julia, M.Sc., M.Res.,[a] Rocio Rivera-Egea, M.Sc., Ph.D.,[d] Ana Navarro-Gomezlechon, M.Sc.,[a] and Nicolas Garrido, M.Sc., Ph.D.[a]

[a] Instituto Valenciano de Infertilidad Foundation, Instituto de Investigación Sanitaria La Fe (IIS La Fe), Valencia, Spain; [b] Andrology Laboratory and Sperm Bank, Instituto Valenciano de Infertilidad Reproductive Medicine Associates of New Jersey Madrid, Madrid, Spain; [c] Alfonso X el Sabio University, Madrid, Spain; and [d] Andrology Laboratory and Sperm Bank, Instituto Valenciano de Infertilidad Reproductive Medicine Associates of New Jersey Valencia, Valencia, Spain

Objective: To better study the effect of sperm deoxyribonucleic acid fragmentation (SDF) on intracytoplasmic sperm injection (ICSI) outcomes from an ovum donation program by assessing the cumulative live birth rates (CLBRs) per number of embryo transfers (ETs), embryos replaced (EmbR), and metaphase II (MII) oocytes required in consecutive treatments to achieve the first newborn.
Design: A multicenter retrospective cohort study was conducted, and the Kaplan-Meier survival curves were generated to calculate the CLBR with regard to the SDF degree.
Setting: Private university-affiliated in vitro fertilization centers.
Patient(s): Data from 864 couples using donated eggs and undergoing ICSI from 2000 to 2019 were analyzed. Sperm deoxyribonucleic acid fragmentation was measured using terminal deoxynucleotidyl transferase biotin dUTP nick end labeling assay on their ejaculated sperm.
Intervention(s): None.
Main Outcome Measure(s): Live birth rate (LBR) per first ET and per all consecutive ETs within the same patient and CLBR per ET, per EmbR, and per MII oocyte used considering the SDF level.
Result(s): A total of 1,903 ICSI cycles were considered, encompassing 6,340 donated oocytes, 2,543 embryos, and 1,145 ETs. Comparing $\leq$15% SDF (low) with >15% SDF (high) or by 10% SDF ranges, the LBRs per first ET and per all ETs did not significantly differ. The Kaplan-Meier curves of the CLBR per ET, per EmbR, and per donor oocyte consumed were similar between the SDF groups evaluated.

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Reprint requests: Irene Hervás, M.Sc., IVI Foundation, Instituto de Investigación Sanitaria La Fe (IIS La Fe), Av. Fernando Abril Martorell, nº106, Torre A, Planta 1ª, 46026 Valencia, Spain (E-mail: irene.hervas@ivirma.com).

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Conclusion(s): Elevated SDF does not reduce the LBR or cumulative probability to obtain a child when calculated per ET, per EmbR, and per donated MII oocyte used in couples undergoing ICSI cycles. (Fertil Steril® 2022;■:■–■. ©2022 by American Society for Reproductive Medicine.)

Key Words: Cumulative live birth rates, donated oocytes, male factor infertility, sperm DNA fragmentation, TUNEL assay

Sperm deoxyribonucleic acid (DNA) fragmentation (SDF) has emerged as one of the most controversial topics relating to male factor infertility. The poor predictive value of semen analysis (1) prompted a search for biomarkers that more accurately assess male fertility potential in couples with fertility issues. One of these approaches measures sperm DNA integrity because of its theoretical importance in transmission of paternal information to the embryo (2). Sperm DNA fragmentation is defined as the presence of single- or double-strand breaks within sperm DNA (3) that may originate from several factors (4), with oxidative stress being one of the main causes (3). High SDF was found in the ejaculated semen of couples undergoing in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (5). In addition, SDF has been detected in both males with normal and abnormal semen parameters (6, 7), but the negative effect of damaged spermatic DNA on reproductive success remains an open discussion.

Sperm DNA damage has been negatively correlated with fertilization and implantation rates (8), delayed embryo development (9), and poor embryo quality (10, 11) in IVF cycles and positively correlated with miscarriages (12–14). In cycles with donated oocytes, high SDF produced a delay in embryo kinetics (15), and patients with ≥15% SDF showed low blastulation and pregnancy rates (per transfer) (16). Furthermore, infertile couples with higher SDF using their own oocytes and undergoing IVF show reduced live birth rates (LBRs) compared with those with low SDF, although this effect was not found in ICSI patients (17). By contrast, additional studies found similar implantation, ongoing pregnancy, and miscarriage rates between the low- and high-SDF groups in ICSI cycles (11, 18), and the level of sperm DNA damage was not statistically different in pregnant and nonpregnant women in IVF cycles using their own eggs and donated eggs (19). The largest review published to date (3) also highlighted the conflicting conclusions of published reports and meta-analyses, studies with the highest scientific evidence.

The current controversy surrounding the influence of SDF on reproductive outcomes may be because of heterogeneity in the male population, lack of controlling for female factors, or method of measuring assisted reproductive technology (ART) success. Clinical outcomes are commonly expressed per embryo transfer (ET) and consider only the best-quality embryos that are chosen first and replaced but do not consider the contribution of the remaining embryo cohort on reproductive success; therefore, information is lost. This induces a significant bias by only comparing the best among the different cohorts head-to-head. A more reliable description and accurate method to measure the impact of SDF on the chances of having a live birth is through the assessment of the cumulative live birth rates (CLBRs). Using this proxy, it is possible to calculate the pace at which live births are obtained and how this may change as failed attempts accumulate.

Previously, our team published several studies assessing the likelihood of a given couple achieving a live birth according to the number of embryos transferred (20, 21) and oocytes used (22–24). Assessment by means of CLBRs can use 3 approaches that provide different information. First, the CLBR can be calculated according to the number of consecutive ETs until the first live birth is achieved. However, this approach does not account for the number of embryos in each transfer. Second, the CLBR can be estimated according to the total number of replaced embryos, providing more accurate information on the number of embryos that each couple needs to transfer. This approach does not penalize cycles where the ET has been cancelled for embryo quality-related reasons. Third, the CLBR can be calculated according to the number of consumed donated oocytes; this approach is a better way to measure and report the yield of the ART cycle and effect of fertilizing spermatozoa. The rationale behind this approach is that the lower the number of eggs needed to reach the first live birth, the better the sperm sample quality, including when using donated oocytes.

To date, no published studies have described CLBRs in the context of high or low sperm DNA damage. This novel approach allows for the assessment of how SDF may influence reproductive outcomes. Furthermore, the use of donor oocytes is an ideal model to assess the effect of SDF on the LBRs because confounding female-dependent factors are minimized (25).

We sought to assess the influence of SDF on the LBRs and measure the success of ICSI cycles from an oocyte donation program according to the CLBR per total number of ETs performed, per total number of consecutive embryos replaced (EmbR), and per total number of donated metaphase II (MII) oocytes consumed until a successful live birth was achieved.

MATERIALS AND METHODS

Study Population

This retrospective cohort study was performed with clinical data from ICSI cycles conducted between January 2000 and March 2019 using donated oocytes from Spanish IVIRMA clinics. Patients from the oocyte donation program who had a terminal deoxynucleotidyl transferase biotin dUTP nick end labeling (TUNEL) assay-based SDF test performed on their

fresh ejaculated sperm and were undergoing a fresh and/or frozen-thawed ET were included.

The study was approved by the Institutional Review Board (project code 1902-FIVI-027-NG).

Clinical indications for SDF testing included couples with previous implantation failures or recurrent miscarriages, males with unknown but long-term infertility, males with very low sperm motility without genital tract infection, and at the patient's request. Sperm DNA fragmentation was described as the percentage of cells with fragmented DNA as a function of the total number analyzed. The study population was divided into groups to perform the analysis. Groups were categorized by SDF (SDF of $\leq$15% [low, $n = 694$] and SDF of >15% [high, $n = 125$]) or by SDF ranges (SDF of <10% [$n = 570$], SDF of 10%–20% [$n = 171$], SDF of 20%–30% [$n = 56$], and SDF of >30% [$n = 22$]).

Oocyte Donors

Oocyte donors were recruited from young, healthy volunteers (18–35 years old), as previously reported (26). All women included in the ovum donation program were screened for sexually transmitted diseases, were in good physical and mental health, had regular menstrual cycles, and did not have a family history of hereditary or chromosomal diseases. The ovarian stimulation protocol and oocyte retrieval were performed following previously described protocols (22). Egg donation was altruistic and anonymous.

SDF Assay

Terminal deoxynucleotidyl transferase biotin dUTP nick end labeling assay was performed to measure the DNA fragmentation of fresh sperm samples (after 3–5 days of sexual abstinence) using the In situ Cell Death Detection Kit (Roche Diagnostics, Barcelona, Spain) according to the manufacturer's instructions at IVIRMA Madrid. The quantification of sperm chromatin integrity was performed as previously published (15), in a minimum of 10,000 sperm cells. The proportion of sperm with fragmented DNA was measured using a FACScan (Becton Dickinson, Franklin Lakes, NJ) cytometer until 2015 and since then using a MACSQuant (Miltenyi Biotec, Bergisch Gladbach, Germany) cytometer.

IVF Laboratory Procedures and ET

Semen samples were collected by masturbation into a sterile recipient after 3–5 days of sexual abstinence. After liquefaction for 30 minutes, semen samples were evaluated for volume, sperm count, motility, and morphology in a Makler chamber (Sefi Laboratories, Tel Aviv, Israel) according to the World Health Organization criteria (27). If necessary, the semen sample was frozen (28) for later use. Semen preparation was performed by density gradient centrifugation (15) or by swim-up (29).

The mature donated ova were fresh or cryopreserved after the vitrification-warming procedures (23). After matching the most adequate egg donor with the recipient according to phenotypic characteristics, the insemination procedure was performed using the ICSI technique, and the sperm were introduced into oocytes (15). All injected oocytes were cultured, and fertilization was confirmed by the presence of 2 pronuclei and 2 polar bodies. Zygotes were cultured until day (D)5 of embryonic development under a controlled environment (37°C, 5% O_2, and 6% CO_2).

Embryos were morphologically evaluated on D3 and D5 of culture using an inverted microscope under ×400 magnification. Cleavage-stage embryos (D3) were assessed according to the Association for the Study of Reproductive Biology, Spain, guidelines (30). The morphological parameters evaluated were the number of blastomeres, cell symmetry, percentage of fragmentation, and presence of multinucleation. An optimal cleavage-stage embryo was defined as one with 7–10 cells on D3, type 1 symmetry blastomeres, and <10% fragmentation and without multinucleation. Embryos lacking any of these characteristics were considered low-quality embryos.

Embryos that reached blastocyst stage were evaluated according to the Association for the Study of Reproductive Biology classification (31). The morphologies of the trophectoderm and inner cell mass were examined to classify embryo quality from A to D according to the embryologists' criteria, where types A and B were good-quality and types C and D were low and poor quality, respectively. Good-quality embryos were considered for transfer or for vitrification (32).

Recipients had a fresh ET or a frozen-thawed ET depending on their requirements either after a spontaneous ovulatory cycle or after hormone replacement therapy for endometrial preparation. The endometrial preparation protocol has been described elsewhere (33). The ET took place between 2 and 6 days of embryo development, and the best embryo was chosen according to the morphological criteria. The number of embryos to transfer complied with national regulations and responded to the needs, requests, and possibilities of the patient.

Clinical Data Collection

The laboratory outcomes evaluated were optimal D3 embryo rate (number of high-quality cleavage-stage embryos divided by the number of oocytes used), blastocyst rate (number of embryos reaching blastocyst stage at D5 divided by the number of oocytes used), and good-quality blastocyst rate (number of A and B blastocysts divided by the number of oocytes used).

Clinical outcomes assessed included the implantation rate (ratio of the numbers of gestational sacs and transferred embryos), chemical pregnancy rate (serum β-human chorionic gonadotropin level of >10 UI 14 days after ET), clinical pregnancy rate (detection of fetal heartbeat by transvaginal ultrasound 21 days after ET), and ongoing pregnancy rate (defined as a pregnancy of >12 weeks of gestational age). The miscarriage rate was defined as the absence of intrauterine pregnancy after a positive β-human chorionic gonadotropin test result. The clinical pregnancy loss rate was defined as a spontaneous miscarriage before 12 weeks. The live birth rate (LBR) was defined as the number of deliveries of at least 1 newborn. All outcomes were calculated per ICSI cycle.

TABLE 1

Baseline demographic and clinical characteristics of oocyte donors and study subjects according to low (≤15%) or high (>15%) SDF.

	SDF ≤ 15% (n = 1,626)		SDF > 15% (n = 277)		
Variable	**Mean or proportion (n)**	**95% CI**	**Mean or proportion (n)**	**95% CI**	***P* value**
Oocyte donors					
Antral follicular count	21.4 (1,371)	21.1–21.7	20.9 (214)	19.9–21.8	.36[a]
No. of stimulation d	10.6 (1,620)	10.5–10.7	10.6 (272)	10.4–10.7	.74[a]
E2 level at hCG trigger (IU)	2,783.4 (1,536)	2,687.8–2,879.0	2,675.3 (261)	2,488.5–2,862.1	.31[a]
P4 level at hCG trigger (IU)	0.9 (189)	0.8–1.1	1.1 (215)	0.7–1.4	.53[a]
No. of aspirated oocytes	20.2 (1,625)	19.8–20.6	20.5 (276)	19.5–21.5	.49[a]
Patients					
SDF index (%)	5.9 (1,626)	5.7–6.1	24.3 (277)	23.2–25.3	<.001[a]
Male age (y)	41.6 (1,586)	41.4–41.9	41.8 (268)	41.2–42.4	.62[a]
Male BMI (kg/m^2)	22.9 (1,594)	22.8–23.1	23.3 (263)	22.8–23.7	.22[a]
Female age (y)	40.4 (1,626)	40.2–40.6	40.8 (277)	40.3–41.2	.14[a]
Female BMI (kg/m^2)	23.0 (1,221)	22.8–23.2	23.4 (169)	22.7–24.0	.23[a]
Oocyte state (n)					.03[b]
Fresh (%)	48.3% (740/1,537)	45.8–50.8	56.2% (136/242)	50.0–62.5	–
Vitrified (%)	51.7% (792/1,537)	49.2–54.2	43.8% (106/242)	37.6–50.1	–
Days of endometrial preparation	22.6 (1,353)	22.0–23.2	23.0 (215)	21.4–24.5	.80[a]
Final endometrial thickness (mm)	9.2 (1,371)	9.1–9.3	9.2 (217)	9.0–9.4	.92[a]
Endometrial preparation protocol (n)					.27[b]
Hormone replacement therapy (%)	93.7% (1,498/1,598)	92.5–94.9	95.6% (262/274)	93.2–98.0	–
Natural cycle (%)	6.3% (100/1,598)	5.1–7.5	4.4% (12/274)	2.0–6.8	–
Semen state					.55[b]
Fresh (%)	82.7% (1,343/1,624)	80.9–84.5	81.2% (224/276)	76.6–85.8	–
Frozen (%)	17.3% (281/1,624)	15.5–19.1	18.8% (52/276)	14.2–23.4	–

Note: CI = confidence interval; BMI = body mass index; E2 = estradiol; hCG = human chorionic gonadotropin; P4 = progesterone; SD = sperm deoxyribonucleic acid fragmentation.
[a] Student's *t* test.
[b] Fisher's exact test.

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Statistical Analysis

Statistical analysis was performed using R software (4.02 version). A 2-sided P value of $<.05$ was considered statistically significant. The study population was divided into groups according to the SDF level, which was treated as a categorical variable. Demographic and clinical parameters were calculated by means or proportions and their corresponding 95% confidence interval (95% CI) and were compared using Student's t test, the chi-square test, and Fisher's exact test, as appropriate. The odds ratio (OR [95% CI]) of the LBR was calculated in terms of the SDF value and was always compared with the lowest value with Fisher's exact test.

The cumulative probability of having a live birth was calculated using the Kaplan-Meier method according to the total number of ETs, number of consecutive EmbR, and number of MII oocytes used until a live birth was achieved. All CLBR data are presented as CLBR (95% CI). Survival curves were categorized by SDF level and compared using the Mantel-Cox test. All patients who started a treatment with transferred embryos were considered, including all attempts of both fresh and frozen-thawed ETs until a live birth was achieved and those who were unsuccessful (all embryos were transfected, but no live births were achieved). Subsequent attempts by couples after a failed first ICSI attempt were counted; however, those who returned to achieve a second pregnancy were not included. To calculate the number of MII oocytes used until a live birth, only the donated oocytes that were microinjected and resulted in a transferred or nonviable embryo were considered. All oocytes and embryos that were vitrified at the time of data export were not included.

Clinical factors that may simultaneously impact the likelihood of a live birth were analyzed using a proportional-hazards model (Cox model) considering the CLBR as the dependent variable and the number of ETs, EmbR, and oocytes used as the independent variables. The variables included in the model were chosen on the basis of their clinical relevance in ART. The covariates assessed were SDF, age (both women and men), body mass index, semen state, controlled ovarian stimulation, protocol, and ET day. A tendency of association of the covariates with the CLBR was confirmed as a P value of $<.05$.

Receiver operating characteristic (ROC) curve analysis was performed to assess the predictive value of SDF on the achievement of a live birth in ICSI cycles. The results are expressed as area under the curve (AUC).

RESULTS

Our cohort consisted of 864 couples who underwent 1,903 ICSI procedures. A total of 6,340 donated MII oocytes were used, resulting in 2,543 transferred embryos (1,552 fresh and 991 frozen-thawed). A mean number of 2.1 embryos were replaced per patient, and an average of 1.4 embryos were used per transfer. Of these, 678 were deliveries with at least 1 live birth (n = 821 newborns). The overall SDF mean was 8.6% (95% CI, 8.2–8.9; range, 1%–70%). The main

indications for oocyte donation were advanced maternal age (66.6%), poor ovarian response (12.8%), polycystic ovarian syndrome (5.1%), and endometriosis (2.6%).

The descriptive analysis of the patient demographics and the donor controlled ovarian stimulation-related parameters for the different SDF categories are shown in Table 1. Variables were equally distributed among groups. The comparative analysis of seminal parameters according to SDF is shown in Supplemental Table 1 (available online).

Table 2 provides the cycle outcomes comparing SDF below or above 15%. Supplemental Table 2 (available online) shows the ICSI outcomes considering a cutoff value of 20% SDF (as extra information). No deleterious effect of high fragmentation was observed in the LBRs. The LBRs per first ET were 48.2% (44.4–52.0) and 53.6% (44.3–62.9) for the ≤15% and >15% SDF groups, respectively (OR, 1.3 [0.8–1.9]; $P=.3$). When categorized by ranges, the LBRs were not statistically different ($P=.3$): SDF of <10%, 48.7% (44.5–52.9); SDF of 10%–20%, 45.5% (37.7–53.3; OR, 0.88 [0.6–1.3]; $P=.5$); SDF of 20%–30%, 58.0% (44.3–71.1; OR, 1.5 [0.8–2.8]; $P=.3$); and SDF of >30%, 61.1% (38.6–83.6; OR, 1.7 [0.6–5.1]; $P=.3$).

Differences in the LBRs per all ETs performed were not statistically significantly different between the low- and high-SDF groups (39.6% [37.1–42.1] vs. 43.3% [37.0–49.6]; OR, 1.2 [0.9–1.6]; $P=.3$) and between SDF ranges (<10% SDF, 39.4% [37.5–41.3]; 10%–20% SDF, 39.2% [34.5–43.9] [OR, 1.0 {0.8–1.3}; $P=1.0$]; 20%–30% SDF, 52.0% [43.0–61.1] [OR, 1.66 {1.1–2.6}; $P=.02$]; and >30% SDF, 38.3% [26.5–50.1] [OR, 1.0 {0.5–1.8}; $P=1.0$]).

CLBR According to ET

When calculating the CLBR according to the number of ETs until a live birth was achieved (Supplemental Fig. 1, available online), there were no statistically significant differences between the ≤15% and >15% SDF groups: 47.7% (43.8–51.3) vs. 52.9% (43.1–60.9) at the first ET and 66.7% (62.7–70.2) vs. 71.5% (61.7–78.8) at the second ET, reaching 83.0% (78.8–86.3) and 82.3% (71.6–89.0) when up to 4 ETs were performed.

If computed by SDF ranges, the CLBR of the group with the lowest vs. the highest SDF was not statistically different in the first 3 consecutive transfers: 48.4% (44.2–52.4) vs. 54.6% (28.2–71.2); 67.0% (62.6–70.8) vs. 74.8% (47.0–88.0); and 75.4% (71.0–79.1) vs. 81.1% (51.8–92.6), respectively. The Cox regression model showed a significant relationship between SDF and the CLBR (hazard ratio [HR], 1.4; 95% CI, 1.1–1.9; $P<.05$).

The AUC value of the ROC curve was 58.1% (95% CI, 54.8–61.3; $P<.001$).

CLBR According to EmbR

Relative to the CLBR comparing the ≤15% vs. >15% SDF groups, the rates of 55.9% (51.9–59.5) vs. 59.4% (49.5–67.3) were reached with 2 EmbR. The CLBRs were 73.4% (69.3–76.9) vs. 79.0% (68.6–86.0) when up to 4 EmbR were performed and 84.3% (80.2–87.5) vs. 85.2% (73.4–91.8) when 6 EmbR were performed. A higher CLBR was found in the ≤15% SDF group than in the >15% SDF group (91.4% [87.2–94.3] vs. 88.9% [74.9–95.1]) when up to 8 EmbR were performed. This was not statistically different (Fig. 1A).

When calculated by SDF ranges (Fig. 1B), comparing the lowest with the highest group, with 2 EmbR, the LBR was 57.3% (52.9–61.2) vs. 61.5% (33.8–77.6), and with a total of 4 EmbR, the LBR was 73.5% (69.1–77.4) vs. 83.5% (54.6–94.0) ($P=.6$). The Cox model analysis showed a significant relationship between the SDF and CLBR (HR, 1.5 [95% CI, 1.1–1.9]; $P<.01$).

The ROC curve analysis showed an AUC of 58.0% (95% CI, 54.8–61.3; $P<.001$).

TABLE 2

Laboratory and clinical outcomes per cycle according to the SDF groups (≤15% or > 15%).

Variable	≤15% SDF (*n* = 1,626)	95% CI	> 15% SDF (*n* = 277)	95% CI	*P* value[a]
Laboratory outcomes					
Optimal cleavage-stage embryo rate	21.7%	19.0–24.5	21.1%	13.9–28.3	.9
Blastocyst rate	49.8%	48.0–51.5	53.4%	48.8–58.1	.1
Good-quality blastocyst rate	24.8%	23.6–25.9	23.5%	20.9–26.2	.4
Clinical outcomes					
Implantation rate	86.9% (777)	85.1–88.7	86.6% (133)	82.5–90.7	.89
Mean no. of sacs	1.2 (777)	1.18–2.24	1.3 (133)	1.2–1.3	.28
Biochemical pregnancy rate	58.3% (1,509)	55.8–-60.8	65.0% (254)	59.1–70.9	.04
Clinical pregnancy rate	50.9% (1,509)	48.4–53.4	55.5% (254)	49.4–61.7	.17
Ongoing pregnancy rate	41.5% (1,500)	39.0–44.0	45.8% (249)	39.6–49.6	.21
Miscarriage rate	6.7% (1,509)	5.4–8.0	8.7% (254)	5.2–12.1	.29
Clinical pregnancy loss rate	9.1% (1,500)	7.6–10.5	8.8% (249)	5.3–12.4	.91
Live birth rate	36.9% (1,453)	37.1–42.1	43.3% (238)	36.9–49.6	.29
Singleton	31.1% (452/1,453)	28.7–33.5	35.7% (855/238)	29.6–41.8	.55
Twins and triplets	8.4% (123/1,453)	7.0–9.8	7.6% (18/238)	4.2–11.0	

Note: Values are presented as mean or proportion (*n*) with 95% confidence interval (95% CI) calculated per cycle. CI = confidence interval; SDF = sperm deoxyribonucleic acid fragmentation.
[a] *P* values were calculated using Student's *t* test.

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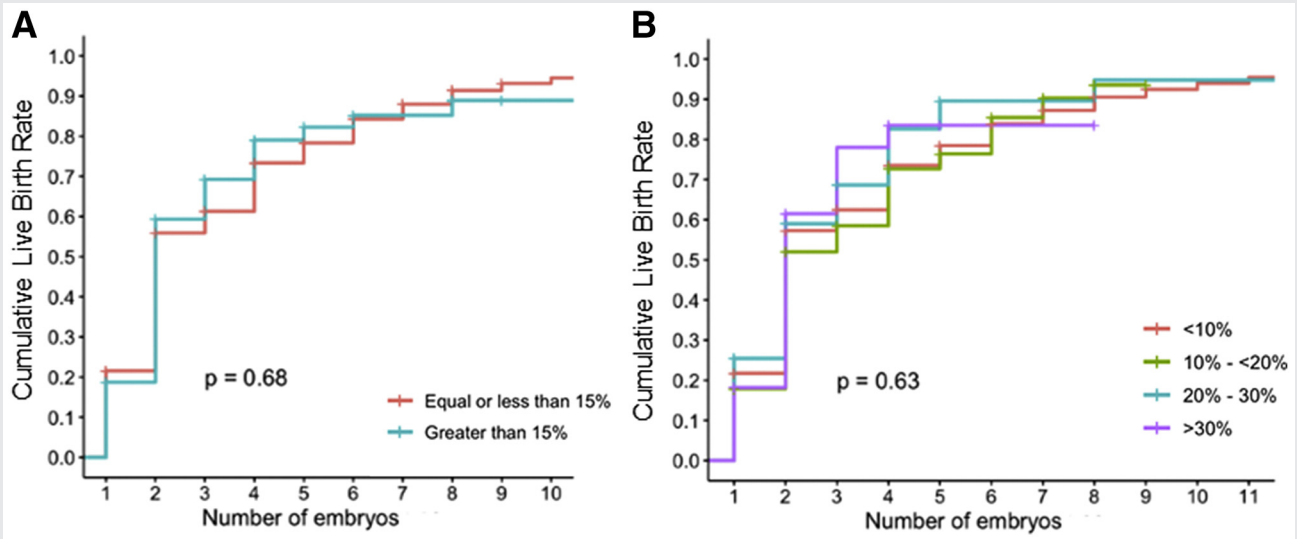
FIGURE 1



Cumulative live birth rates (CLBRs) according to the total number of consecutive embryos replaced. Kaplan-Meier curves plotting the CLBR depending on the number of embryos replaced until a live birth was achieved. Data are categorized by (**A**) high or low sperm deoxyribonucleic acid fragmentation and (**B**) sperm deoxyribonucleic acid fragmentation groups. Data were analyzed using the Mantel-Cox test.

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CLBR According to MII Oocytes Used

When 5 MII oocytes were used, the CLBRs were similar between the ≤15% and >15% SDF groups (Fig. 2A), with rates of 42.5% (38.8–46.4) in the former group and 45.9% (36.1–54.2) in the latter group. When up to 10 MII oocytes were used, the CLBRs were 78.6% (74.6–82.0) and 82.0% (71.6–88.6), respectively, representing an increase of 7.2% per additional oocyte in both cases. When up to 15 oocytes were used, the CLBR was higher in the ≤15% SDF group (91.8% [82.0–94.4]) than in the >15% SDF group (87.6% [78.4–95.2]). In patients requiring 19 oocytes, the maximum CLBRs were 96.3% (93.8–98.1) in the lower-SDF groups and 93.1% (79.6–97.7) in the higher-SDF group, which was slightly higher than the previous rate. These differences were not statistically significant.

Differences were not statistically significant when comparing between SDF ranges (<10% vs. >30% SDF) (Fig. 2B). The CLBR was not reduced by high fragmentation when 5 oocytes were used (44.2% [39.9–48.3], low SDF, vs. 38.1% [13.4–55.7], high SDF) and when 10 oocytes were used (79.4% [75.0–83.0], low SDF, vs. 87.6% [56.4–96.5], high SDF), with rates of increase of 7.4% and 9.9% CLBR per oocyte used in this category, respectively. In patients who needed 15 oocytes, the CLBR attained over 90% probability of having a child. The estimated hazard of the Cox model analysis showed a significant relationship between SDF and CLBR (HR, 1.32; 95% CI, 1.0–1.7; $P=.04$).

The ROC curve analysis did not show that SDF had any predictive value on the LBR (AUC, 55.6%; 95% CI, 53.7–57.4; $P<.001$).

DISCUSSION

In this retrospective study, we observed that SDF does not diminish the LBR or reduce the success of ICSI cycles of undifferentiated couples when evaluating per ET or through CLBRs per transferred embryo and per oocyte used in our oocyte donation program. To our knowledge, this is the first study evaluating the effect of high SDF measured using TUNEL assay on the CLBRs. This method provides a better and more reliable assessment of the impact of sperm-related factors on ICSI cycle's success with donor oocytes.

Currently, conventional semen analysis is unable to evaluate the inner physiological features of the spermatozoon that could jeopardize cycle success in couples with initially good prognosis. Sperm DNA fragmentation has recently been highlighted for its potential biologic implications in fertility. Damaged paternal chromatin may cause failures in early embryo development, both in vitro and in the womb, as long as genes essential for the growth of the embryo and the future child could be compromised (34).

Nowadays, it is not yet known whether DNA fragmentation is the origin or the result of poor semen quality. In our study population, the group of males with an SDF of >15% presented worse seminal parameters in terms of sperm concentration, motility, and total sperm count than their peers, although these were within the normal World Health Organization standards (27). Worsening of semen quality when DNA fragmentation is present was reported previously as well (11, 35, 36). However, the association between elevated SDF and poor pregnancy outcomes remains controversial despite the number of studies conducted. A recent review (37) indicated

an overall negative effect of SDF on pregnancy rates and an increased risk of miscarriages in IVF and ICSI cycles. By contrast, a meta-analysis of 5 studies (397 patients) did not find such a relationship (38). However, the heterogeneity in study design and subject populations makes it difficult to interpret and generalize the results obtained in routine clinical practice.

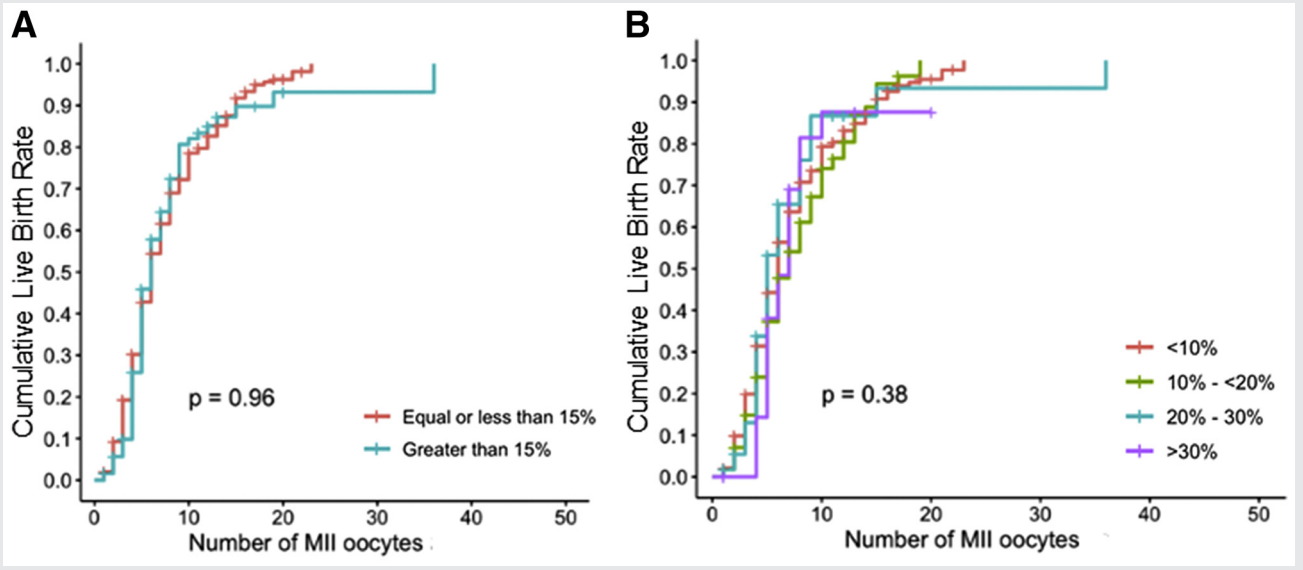
Up to now, previous studies showed a negative effect of SDF on embryo development and quality after the cleavage-stage (commonly known as late paternal effect (39)) in couples undergoing ICSI cycles (11, 15). However, this phenomenon was not observed in our study where couples with an SDF of >15% using young and healthy oocytes had the same proportion of good-quality embryos on D3 and D5, which is in accordance with previous findings in euploid embryos from donated eggs (36).

Furthermore, we observed that an SDF of >15% was not associated with poorer pregnancy outcomes after ICSI. However, it was marginally associated with an increased rate of miscarriages. These results are in agreement with previous studies where the fertilization, implantation, and ongoing pregnancy rates were similar between the low- and high-SDF groups measured using the sperm chromatin dispersion test and TUNEL assay in donor egg ICSI cycles (11, 15). By contrast, in another study performed in one of our centers using TUNEL assay, SDF was not related to ICSI outcomes with donated oocytes, and no differences were found in the SDF levels between pregnant and nonpregnant couples (19). Additionally, a latest study reported that SDF measured using TUNEL assay does not affect ART outcomes regardless of semen quality and oocyte origin (40). Several studies have confirmed an increased chance of pregnancy loss after IVF/ICSI in couples with SDF when autologous oocytes are used (8, 12, 14). However, very few studies evaluated the miscarriage probability in couples with SDF but using donor's oocyte. Similar to our results, a higher rate of miscarriage was observed in couples using donated eggs and who had an SDF of >20% by TUNEL assay than in couples with low SDF (15), even though this was not statistically significant in our study.

Achieving a live birth is ultimately the goal of all patients undergoing ART but, surprisingly, little information is available in this issue. In 1 study, elevated SDF tested using alkaline comet assay reduced the LBR of couples who underwent IVF but not ICSI (17). The same finding was described in 1 meta-analysis (41), which reported a significantly higher LBR in couples with lower-SDF in IVF cycles but only a small relationship in ICSI cycles. Conversely, a major meta-analysis of 1,785 couples revealed that there were no statistically significant differences between the low- and high-SDF groups in the LBRs (42). In our study, we also did not find that an SDF of >15% reduced the LBR per ICSI cycle, per first ET, and per all ETs performed.

However, this traditional measurement per ET does not address the effects of sperm-related factors on the cycle's embryo cohort. Representing the success rates by CLBR per analysis unit according to SDF degree provides a better assessment of cycle performance. In addition, conducting the analysis with donated oocytes allows for a much clearer evaluation of the effect of the male gamete on the cycle by standardizing the female factor. The CLBR per oocyte has previously been used as a method to determine how female-related factors (23, 43) or sperm-related factors (24, 44, 45) affect cycle success because each inseminated egg until a live birth was achieved by the couple is counted. This design provides useful

FIGURE 2



Cumulative live birth rates (CLBRs) according to donated metaphase II (MII) oocytes used. Kaplan-Meier curves plotting the CLBR depending on the number of donated MII oocytes used until a live birth was achieved. Data are categorized by (**A**) high or low sperm deoxyribonucleic acid fragmentation and (**B**) sperm deoxyribonucleic acid fragmentation groups. Data were analyzed using the Mantel-Cox test.

Hervás. SDF and cumulative live birth rates. Fertil Steril 2022.

information from the beginning of embryonic development. However, the CLBR per EmbR (20, 21) only considers the embryos that were consecutively transferred from the same oocyte donation cycle but does not contemplate embryos that were obtained but blocked at early stages before transfer.

These results reached revealed that the existence of damaged paternal chromatin does not worsen the cumulative probability of a successful pregnancy when oocytes from young healthy donors were used in nonselected couples employing TUNEL assay. Interestingly, when the analysis was performed in terms of 2 groups ($\leq$15% or >15% SDF), the group with >15% SDF had a higher CLBR than those with low fragmentation when up to 15 MII oocytes were used (which is normally a complete oocyte donation cycle). Additionally, the second CLBR analysis considering 4 different fragmentation degrees allowed us to evaluate the impact of higher SDFs, which affords much more useful information than only analyzing the results according to a single cutoff. In this additional proxy, a negative effect was also not seen when the same number of donated MII oocytes was used in groups with an SDF of >20%. Even so, the 15% cutoff value was chosen to make a balanced statistical analysis between the 2 groups, which has also been used previously (3, 16, 18) although the tests applied were different.

We also controlled for potential confounding factors because of the high variability of retrospective data compressing such a long period of time. A significant relationship was found in the Cox analysis between SDF and the LBR, meaning that the higher the level of fragmentation, the higher the probability of having a child. This result agrees with the previous univariate analysis, although in the latter analysis, the difference was not statistically significant. This finding contradicts the evidence published so far, in which the presence of sperm fragmentation suggests poor reproductive outcomes in couples requiring ART. A possible explanation for this result is that the harmful effects of damaged paternal chromatin on the embryo are not as deleterious when the quality of the oocyte is optimal (as occurs when oocytes from young, healthy donors are used). It is well known that sperm do not have the necessary machinery to repair DNA breaks, yet eggs do. Donated oocytes ultimately have higher quality and, therefore, higher repair capabilities (46). Thus, we hypothesize that oocytes can use extra energy when there is a higher level of fragmentation, resulting in more competent embryos and higher LBRs. However, from our analysis, we cannot determine what SDF level is too high for the egg to compensate. Another reason behind these results may be the small sample size of the >20% and >30% SDF groups, limiting the statistical power to find a significant relationship and perhaps diluting the effect size of elevated SDF on clinical outcomes. Apart from this, the use of the ICSI technique has been proven to suppress the negative effects that SDF could have on ICSI outcomes, unlike after intrauterine insemination and IVF as reported in the meta-analyses of Osman et al. (41) and Zini (47). Furthermore, the lack of impact of elevated SDF on the ICSI LBRs may be related to the previous sperm selection for motility and morphology regarding the whole semen sample, associated with a lower proportion of single-strand DNA breaks (48). This could explain the lack of effect of SDF on ICSI clinical outcomes between the most extreme groups. More well-designed and randomized studies are required that further evaluate the role of SDF when ICSI is used.

Finally, the estimation of the AUC value for each model revealed that SDF by TUNEL assay has a poor predictive value for the achievement of a live birth after ICSI using donated oocytes. Therefore, paternal DNA fragmentation greater or less than 15% as measured using TUNEL assay would not be a useful predictor for the attainment of a newborn.

This study presents some weakness. Importantly, the SDF degree was not measured on the sample used for fertilization but on a different sample produced closest to the day of ICSI used for diagnosis. Because the level of fragmentation, as well as semen quality, is not stable over time (49), this minimizes the reliability of the prognosis of its effects on ICSI outcomes. The vast time period retrospectively assessed in this study allows, on the one hand, for the construction of a large database, but, on the other, it also introduces heterogeneity bias related to clinical practice. Thus, the CLBR analysis was adjusted so that different confounding factors could be accounted for. Although the multivariable analysis did not consider the status of the oocyte (fresh or vitrified), it has been previously reported that the vitrification protocol does not affect clinical outcomes (50). Another weakness is that we have no record of whether the male who presented an elevated SDF underwent any type of intervention to improve it (e.g., oral antioxidant intake). This may constitute a bias because it could improve the quality of the seminal sample used in the subsequent ICSI, detracting from the veracity of the data presented.

In summary, in this retrospective study we observed that SDF did not adversely affect the LBR or reduce the success of ICSI cycles with donated oocytes of couples attending a reproductive center. Elevated SDF values measured using TUNEL test does not undermine the cumulative likelihood of achieving a live birth when assessed per transferred young embryo or per donor oocyte used in this undifferentiated population. The measurement of ART success by CLBR in ICSI cycles of the oocyte donation program provides more precise and accurate information about sperm with fragmented DNA performance. The information obtained in this study may enhance aid decision-making for clinicians when a couple requires infertility treatment with donor eggs and the male presents elevated DNA fragmentation in his ejaculated sperm. Nonetheless, these conclusions should be interpreted with caution in the different clinical scenarios where SDF testing is performed with another technique and in specific couples presenting SDF-related risk factors.

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