Double-stranded sperm DNA damage is a cause of delay in embryo development and can impair implantation rates

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Objective: To analyze the effect of single- and double-stranded sperm DNA fragmentation (ssSDF and dsSDF) on human embryo kinetics monitored under a time-lapse system.
Design: Observational, double blind, prospective cohort study.
Setting: University spin-off and private center.
Patient(s): One hundred ninety-six embryos from 43 infertile couples were included prospectively.
Intervention(s): None.
Main Outcome Measure(s): SsSDF and dsSDF were analyzed in the same semen sample used for intracytoplasmic sperm injection. Embryo kinetics was then monitored using time-lapse technology, and the timing of each embryo division was obtained.
Result(s): When comparing embryos obtained from semen samples with low dsSDF and high dsSDF, splitting data using a statistically significant delay in high dsSDF was observed in second polar body extrusion, T4, T8, morula, and starting blastocyst and embryo implantation rates were impaired. Embryo kinetics and implantation rates are not significantly affected when high values of ssSDF are present. Different patterns of delay in embryo kinetics were observed for these different types of DNA damage: dsSDF caused a delay along all stages of embryo development; however, its major effect was observed at the second polar body extrusion and morula stages, coinciding with embryo DNA damage checkpoint activation as described before; ssSDF had its major effect at the pronucleus stage, but embryo kinetics was then restored at all following stages. The results show that dsSDF could be the main type of DNA damage that affects embryo development in intracytoplasmic sperm injection cycles, probably due to motility-based sperm selection in this assisted reproduction procedure.
Conclusion(s): Double-stranded sperm DNA damage caused a delay in embryo development and impaired implantation, while single-stranded DNA damage did not significantly affect embryo kinetics and implantation. (Fertil Steril® 2019;111:699–707. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Sperm, embryo kinetics, DNA damage, implantation, male factor

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Standard methods to assess embryo quality are based on intermittent evaluation of parameters such as cell number, cell fragmentation, symmetry, and embryo compaction (1). In a scenario where less than half of intracytoplasmic sperm injection (ICSI) cycles show implantation (2), laboratories have made efforts using technology to perform the best embryo selection to achieve implantation. In addition, technologies such as time-lapse recording

have been implemented in a vast number of assisted reproductive technology (ART) centers. Thanks to the time-lapse system, embryo evaluation has turned from discrete to dynamical observation, which allows both clinicians and researchers to perform better embryo evaluation throughout the entire preimplantation development (1,3–5). Different studies analyzing embryo kinetics pointed out that the timing of embryo cleavage might be an important parameter defining the embryo implantation potential. This fact allowed the generation of mathematic algorithms to predict the best embryo to transfer (4, 6). However, the effectiveness of these models could be altered by multiple cofounding factors (7), evidencing the necessity of validating them in an independent set of samples to prove their utility (3, 8). Research on biomarkers with high predictive power in ART success and new embryo selection criteria is a topic of high interest in clinical practice. With respect to male factor, studies in ICSI cycles show that traditional semen analysis (sperm concentration, motility, and morphology) is not predictive of ICSI implantation rates (9). The introduction of sperm DNA fragmentation (SDF) techniques seemed promising as complementary parameter for the prediction of ART success. Different research groups have performed studies analyzing the predictive power of SDF using different techniques (TUNEL, sperm chromatin structure assay, and sperm chromatin dispersion tests). Some studies showed a relation between DNA damage and implantation rates in ICSI (10–13), but others showed opposite results (14–18). These unclear results could be related to the bias between ejaculate analysis and the selection of a motile sperm cell before realization of ICSI (19, 20), as it is known that a negative correlation is present between sperm motility and DNA fragmentation (21–23).

In recent years, studies analyzing the clinical effect of different types of sperm DNA damage showed that single-strand SDF (ssSDF) detected by alkaline Comet assay is a type of extensive DNA damage that is related to natural pregnancy achievement (24, 25); Comet assay shows good correlation with the TUNEL, sperm chromatin structure assay, and sperm chromatin dispersion tests (26). Alternatively, DNA breaks detected using neutral Comet correspond to matrix attachment region-specific double-stranded DNA damage (24–26) that is related to higher miscarriage risk. These highly localized DNA breaks do not show a correlation with the prior techniques or sperm motility. In animals, physiological studies have shown that induced double-strand breaks in mouse sperm cells cause complex paternal chromosomal reorganizations at the male pronucleus, showing a delay in first embryo DNA replication (27). Other studies inducing sperm double-strand breaks through radiation identified embryo checkpoints related to p53 and p21 in response to paternal double-stranded DNA damage, and a fewer number of fetuses were found when an irradiated sperm sample was used for fertilization (28, 29). Studies analyzing the effect of double-stranded sperm DNA breaks in human ICSI treatments are still emerging (10), but this is a topic that has not been extensively studied.

To the best of our knowledge, only two studies have analyzed the relation between SDF and embryo kinetics during preimplantation development, showing a slower embryo development when high SDF is detected using sperm chromatin dispersion test (30) and TUNEL assay (31). As single- and double-strand DNA fragmentation show different clinical implications in natural pregnancies, the aim of the present study is to analyze the relation of both types of sperm DNA damage and embryo kinetics in ICSI cycles.

MATERIAL AND METHODS

Study Design and Participants

The present prospective and double blind study included data for 196 embryos from 43 infertile couples who attended our center seeking assisted reproductive treatments. The patients included in the study were not under antioxidant treatment. The Parc Taulí Hospital Ethics Committee approved the study (reference no. 2017902).

A semen sample after 3 or fewer days of abstinence was obtained from the patients included in the study on the day of ICSI; a 250 μL aliquot was cryopreserved, and the rest of the sample was used for the ICSI procedure. Sperm concentration and motility were also determined, and single- and double-stranded sperm DNA fragmentation analysis was performed using Comet assay. All the embryos were cultured under a time-lapse monitoring system (Primo Vision, Vitrolife).

Comet Assay: Single- and Double-Strand SDF Analysis

The particular conditions of Comet assay methodology allow the discrimination of single- and double-strand DNA damage. The alkaline Comet protocol described elsewhere (24) and used in the present work mainly evidences the presence of single-strand DNA breaks, while the neutral Comet shows double-strand DNA breaks. Briefly, the semen sample was thawed and washed in phosphate-buffered saline and the sperm concentration was adjusted to $1 \cdot 10^6$ spermatozoa/mL. Then 25 μL of the sample was mixed in 50 μL of melted 1% agarose in distilled water. The mixture was allowed to jellify on two slides at 4°C, and the slides were treated in two consecutive lysis solutions (0.8 M Tris-HCl, 0.8 M DTT, 1% SDS, pH 7.5 and 0.4 M Tris-HCl, 0.4 M DTT, 50 mM EDTA, 2 M NaCl, pH 7.5) for 30 minutes each to remove proteins and unwind sperm DNA. After the lysis step, the slide designated to double-strand SDF (dsSDF) analysis was electrophoresed at 20 volts for 12.5 minutes in tris-borate EDTA buffer at pH 8 and washed in 0.9% NaCl solution. Meanwhile, the slide designated to ssSDF analysis was denatured in NaOH solution at 4°C and electrophoresed at 20 volts for 4 minutes in NaOH at pH 13. Both slides were washed in tris-borate EDTA and dehydrated in ethanol series (70%, 90%, and 100%) for 2 minutes each. Finally, samples were stained using DAPI (Thermo Fisher Scientific), and spermatozoa were scored under epifluorescence microscope (Nikon E200) following the criteria reported elsewhere (24) and depicted in Supplemental Figure 1. Results were expressed as a percentage of fragmented sperm cells. An internal control sample

was repeatedly included in each experiment to control technique variability.

Ovarian Stimulation, Oocyte Retrieval, and ICSI

Patients were stimulated following a personalized protocol that included an initial ovarian stimulation with 150–300 IU FSH (Menopur, Ferring). Doses were calculated on the basis of patient age, body mass index, ovarian reserve, and response in the case where the patient had undergone previous cycles. LH and/or urine gonadotropins were added to the stimulation protocol on the basis of patient age and LH basal values. In most cases, short protocols with GnRH antagonists, triggering with an agonist 34–36 hours before egg collection to initiate ovarian maturation, were used. Cumulus-oocyte complexes were retrieved 34–36 hours post-GnRH trigger injection. Cumulus-oocyte complexes were washed using HEPES medium (LifeGlobal) and cultured in Global fertilization medium (LifeGlobal) in a Labotect C60 incubator (Labotect) at 7.2% CO_2, atmospheric O_2, and 37°C for 2–3 hours before denudation. ICSI was performed 4 hours after egg collection using HEPES medium (LifeGlobal), and injected eggs were placed on a preequilibrated Primo Vision slide (Vitrolife) containing 80 μL of Global medium (LifeGlobal) and a 4 mL overlay of mineral oil (Ovoil, Vitrolife).

Embryo Incubation and Time-Lapse Imaging and Data Acquisition

Embryos were cultured during the 5/6 days after sperm injection in the Primo Vision culture dish at 7.2% CO_2, atmospheric O_2, and 37°C. Embryos were cultured uninterruptedly through the whole development until the blastocyst stage using Global medium.

Time-lapse imaging provided images every 10 minutes for all embryos analyzed. These images were compiled in videos, and one researcher annotated time points corresponding to the following stages of embryo development: second polar body extrusion, pronuclei appearance, pronuclei disappearance, starting first cell division (starting T2), two cells (T2), three cells (T3), four cells (T4), five cells (T5), six cells (T6), seven cells (T7), eight cells (T8), nine cells (T9), morula stage, starting blastocyst, and blastocyst stage. Data obtained are expressed in hours post-ICSI.

Embryo Scoring and Selection

Embryo morphology was evaluated using Gardner's blastocyst classification (32), taking into account the blastocoel expansion, the number of trophectoderm cells, and the inner cell mass. Embryos were vitrified, and one or two best-quality embryos were transferred a month later after a natural cycle that was determined by the LH peak.

Statistics

Data distribution was evaluated using the Kolmogorov-Smirnov test. Comparisons of quantitative variables were performed using the Mann-Whitney *U*-test, since nonparametric analysis was required due to lack of normality. The 95% confidence interval was chosen for statistical significance ($P < .05$) in all statistical tests.

RESULTS

General Data About Patients

Patients' data for low or high ssSDF and dsSDF are displayed in Table 1. Median values were taken as the cut value to discriminate low or high ssSDF and dsSDF. No differences were found in any parameter when comparing couples with high ssSDF and couples with low ssSDF. However, when patients were classified by double-strand DNA damage, the implantation rate was significantly higher in low dsSDF compared with high dsSDF. None of the other parameters showed statistical differences (Table 1).

Single- and Double-Stranded DNA Damage and Progressive Motility

A negative correlation between progressive motility and single-stranded DNA fragmentation has been found ($r = -0.390$; $P = .037$), while no correlation was found between progressive motility and double-stranded DNA damage ($r = -.092$; $P = .642$).

TABLE 1

General data of couples included in the study expressed as median (range) split into groups regarding low or high ssSDF and dsSDF.

	ssSDF			dsSDF		
Variable	**Low**	**High**	***P* value**	**Low**	**High**	***P* value**
Female age, y	37.27 (4.15)	37.31 (3.88)	.458	37.73 (4.45)	37 (3.73)	.508
Female who provides oocytes age	33.59 (6.54)	35.5 (4.59)	.549	34.6 (6.49)	34.26 (5.47)	.719
Male age, y	37.36 (4.57)	39.12 (6.74)	.462	38 (5.50)	38.17 (5.74)	.791
Years of infertility	2.43 (2.50)	3.33 (2.99)	.446	2.57 (2.74)	3.17 (2.79)	.494
Previous pregnancies	0.33 (0.73)	0.47 (0.64)	.323	0.33 (0.49)	0.43 (0.81)	.95
Sperm concentration	57.90 (44.92)	46.94 (53.23)	.474	50.4 (41.95)	55.05 (53.06)	.819
Progressive motility	41.67 (27.03)	34.57 (19.15)	.529	37.87 (26.42)	39.55 (22.95)	.831
Metaphase oocytes retrieved	6.41 (2.50)	7 (2.78)	.988	6.73 (2.74)	6.61 (2.57)	.836
Fertilization rate, %	69	60	.356	67	64	.701
Implantation rate, %	48	24	.102	52	22	.037

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Single-Strand SDF and Embryo Kinetics

Embryo kinetics results were classified according low or high ssSDF (Table 2), expressed in median (range) of hours after fertilization. None of the timings of the developmental stages showed statistically significant results when comparing low ssSDF with high ssSDF samples ($P > .05$; Table 2).

Double-Strand SDF and Embryo Kinetics

Embryo development stages were also classified according to low or high values for dsSDF. The median (range) for every stage is displayed in Table 2. Statistically significant results were found in different stages ($P < .05$): second polar body extrusion, T4, T8, morula, and starting blastocyst.

Single- and Double-Strand Sperm DNA Damage Caused Different Patterns of Delay in Embryo Kinetics

Taking the differences from Table 2, we expressed the relative percentage of embryo delay of the high ssSDF and dsSDF groups in relation to the low ssSDF and dsSDF groups, respectively. Figure 1 displays these differences throughout the process of embryo development. Single-stranded DNA damage shows its major effect at the pronuclei stage, but no increase of delay is shown at other stages. Alternatively, double-stranded DNA damage causes a delay at the extrusion of the second polar body, but the embryo development delay shows a progressive increase as embryo development proceeds. The major effect of double-stranded DNA damage is shown to be at the second polar body stage and between the T9 and morula stages.

Implantation and Embryo Kinetics

We then obtained results for those embryos that were known to have achieved implantation with a positive heartbeat (n = 16) and those that were transferred and did not implant (n = 28). The stages achieved by this small subgroup of embryos are shown in Table 3.

After obtaining the timing of these embryo development stages, they were compared with those from patients with low or high dsSDF as displayed in Table 2. The embryo kinetics of those embryos that achieved implantation was similar to low dsSDF kinetics (mean of 0.4% of difference; $P = .975$) and different from high dsSDF kinetics (mean of 3.8% of difference; $P = .001$). Embryo kinetics from those embryos that did not achieve implantation was similar to high dsSDF kinetics (mean of 1.3% of difference; $P = .670$) and different from low dsSDF kinetics (mean of 5.7% of difference; $P = .001$).

DISCUSSION

In the present study, the effect of single- and double-stranded DNA damage on embryo kinetics, monitored with a time-lapse system, was evaluated. The results suggest that double-stranded DNA fragmentation could be the main type of DNA damage affecting embryo kinetics. Available data showed a delay in embryo kinetics and worse implantation rates when double-stranded DNA damage is increased in the semen sample used for ICSI (Table 1). In contrast, an increase of single-stranded sperm DNA damage did not cause any significant effect either on embryo kinetics or on implantation rates (Table 1). These results may shed light on the effect of different types of sperm DNA damage in embryo development. To the best of our knowledge, this is the first study analyzing different types of sperm DNA breaks and human embryo development.

The effect of SDF on embryo quality and ICSI outcomes has been a controversial topic in recent years, with some studies finding an association between these parameters (10–13) and others showing opposite conclusions (14–18). However, studies analyzing the paternal effect on embryo

TABLE 2

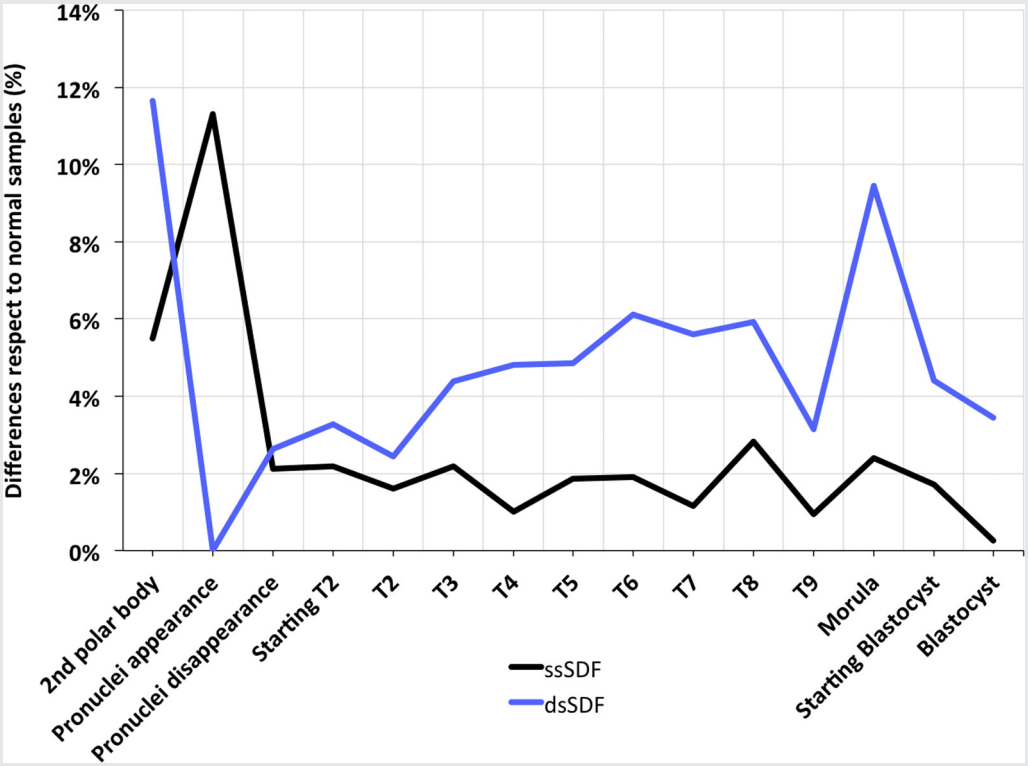
Embryo kinetics classified into low or high ssSDF and dsSDF.

	ssSDF			dsSDF		
Variable	**Low**	**High**	***P* value**	**Low**	**High**	***P* value**
Second polar body	3.02 (13.18)	3.18 (25.66)	.419	2.87 (10.32)	3.20 (26.65)	.008
Pronuclei appearance	8.70 (14.50)	9.68 (23.18)	.06	9.03 (13.32)	9.04 (23.48)	.686
Pronuclei disappearance	24.09 (14.78)	24.60 (31.16)	.155	23.98 (13.87)	24.53 (31.17)	.114
Starting T2	26.25 (15.61)	26.82 (36.56)	.101	25.93 (13.65)	26.63 (36.57)	.127
T2	26.80 (16.95)	27.23 (43.05)	.084	26.59 (14.48)	27.10 (43.05)	.088
T3	38.18 (31.88)	39.02 (45.96)	.195	37.53 (31.88)	39.10 (45.97)	.055
T4	39.85 (33.93)	40.25 (50.80)	.366	38.94 (33.93)	40.56 (50.80)	.043
T5	51.32 (55.41)	52.28 (72.71)	.191	50.77 (55.42)	52.99 (72.72)	.253
T6	53.65 (58.95)	54.67 (76.04)	.173	52.58 (58.60)	54.93 (76.05)	.278
T7	57.82 (46.98)	58.48 (41.71)	.675	55.97 (44.98)	58.48 (44.35)	.255
T8	60.67 (53.48)	62.38 (88.90)	.486	59.10 (51.33)	62.45 (88.90)	.048
T9	70.26 (53.85)	70.92 (63.74)	.879	69.70 (41.95)	71.35 (67.42)	.464
Morula	92.22 (65.01)	94.43 (57.23)	.153	89.15 (56.60)	97.95 (71.38)	.006
Starting blastocyst	105.63 (42.83)	107.45 (45.08)	.095	105.12 (44.98)	109.98 (42.93)	.014
Blastocyst	112.22 (41.53)	112.50 (90.33)	.744	109.72 (41.00)	113.50 (82.90)	.418

Note: Results are expressed as median (range).

Casanovas. Double-strand breaks cause embryo delay. Fertil Steril 2018.

FIGURE 1



Percentage of delay between low and high ssSDF and dsSDF. Single-stranded DNA damage caused a delay at pronuclei appearance, but kinetics was recovered at the next stages. Alternatively, double-stranded DNA damage caused its major delay at second polar body extrusion and morula stages; however, the delay is present throughout the preimplantation embryo development.

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development may show different cofounding factors based on oocyte quality (13) or the technique used for fertilization (33). The consensus is that oocytes may have the ability to repair some of the paternal DNA breaks, depending on quality (19, 34–39). In this sense, a large study of female factors would be desirable to elucidate the effect of male factor, and this may be a limitation for any male-factor study. In fact, in the present study we confirmed that no differences were present between DNA fragmentation groups regarding oocyte age, years of infertility, previous pregnancies, and metaphase II oocytes retrieved (Table 1); however, more parameters such as body mass index or FSH levels would have been interesting to include.

The results showed that single-stranded DNA breaks were not associated with a delay of embryo kinetics at any stage (Table 2). This fact may be explained by the negative correlation found between progressive motility and ssSDF measured by alkaline Comet assay and other techniques measuring SDF (21–23). Taking into account this correlation, and knowing that all sperm cells selected for ICSI show good motility, one would expect that most ICSI selected sperm cells would not show a high amount of single-stranded DNA breaks regardless of ejaculate ssSDF. In fact, this strong bias introduced by the sperm selection in ICSI cycles was previously described in studies trying to find associations between DNA integrity and embryo quality (19, 20). More research is necessary to clarify the effect of oxidative DNA breaks on embryo development.

Regarding double-stranded DNA breaks, an association with delay has been found at different stages of embryo development, including second polar body extrusion, T4, T8, morula, and starting blastocyst (Table 2). Following the hypothesis explained above, dsSDF does not show a correlation with progressive motility; therefore, one would expect that the proportion of positive dsSDF in ICSI-selected sperm cells and the ejaculate should be similar. Then dsSDF would be the predominant DNA damage in ICSI-selected sperm cells and this could explain the associations found between dsSDF and embryo kinetics. From studies in somatic cells, it is well known that double-stranded DNA breaks trigger the initiation of DNA repair machinery and/or apoptosis (40, 41) and a misrepair of a double-strand break is the previous step to chromosome reorganizations, loss of chromosomal fragments, and/or complex reorganizations (42–46). In reproduction, these processes may occur in a similar way during gametogenesis and embryo development (47–49), as it is known that chromosome reorganizations may be present in germ cells, causing a higher risk of miscarriage and infertility (24, 50–52). Thus, we previously observed that alterations of dsSDF are related to a higher risk of recurrent

TABLE 3

Embryo kinetics for transferred embryos according to whether they achieved implantation or not.

Variable	Implantation (n = 16)	No implantation (n = 28)
Second polar body	3.03 (1.82)	3.46 (25.03)
Pronuclei appearance	9.03 (8.55)	10.73 (21.70)
Pronuclei disappearance	24.22 (8.98)	24.40 (13.40)
Starting T2	26.65 (8.98)	26.77 (21.82)
T2	27.02 (8.45)	27.23 (30.05)
T3	38.12 (10.50)	38.90 (36.83)
T4	38.45 (12.02)	38.89 (34.00)
T5	51.32 (24.82)	53.35 (38.00)
T6	52.52 (15.68)	55.82 (50.72)
T7	54.27 (14.37)	56.46 (42.70)
T8	56.12 (13.83)	59.21 (56.00)
T9	69.07 (18.92)	70.63 (43.15)
Morula	91.87 (24.07)	100.35 (44.98)
Starting blastocyst	105.42 (29.05)	111.07 (23.27)
Blastocyst	108.55 (27.72)	113.93 (14.43)

Note: Results are expressed as median (range).

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pregnancy loss in couples experiencing natural pregnancy (25). During embryo development in ICSI treatments, embryos may accumulate structural chromosomal alterations from a paternal origin, leading to a slower development to blastocyst. In fact, aneuploidy has been described as one factor causing delay in embryo development (6, 53–55). Moreover, the presence of blastomere multinucleation, related to implantation rates (56), supports the presence of chromosomal alterations. This multinucleation may resemble the micronuclei that appear when chromosomal aberrations due to double-strand breaks occur in somatic cells (57–59).

The embryo kinetics shown in Figure 1 present different patterns of delay: while the sperm cells from samples with high single-stranded DNA damage have their major effect at pronuclei appearance, double-stranded DNA damage causes an initial delay after fertilization, which is restored, and then a progressive increase of delay is observed until reaching the morula stage (Fig. 1). On the one hand, ssSDF may cause defects of DNA replication at the pronuclear stage because its extensive nature affects the whole genome (25). If a spermatozoon containing single-stranded DNA breaks is used for fertilization, the zygote may induce single-stranded DNA repair before DNA replication in an efficient manner, since the complementary DNA strand is present (29, 34, 60, 61). Therefore, DNA repair might be the reason why embryos do not present delays after first cleavage, and ssSDF may not have a more serious effect on embryo development.

On the other hand, double-stranded DNA breaks in sperm cells are localized mainly at matrix attachment regions (25), and both DNA ends have been demonstrated to be attached at the nuclear matrix (62), which is inherited at the male pronucleus until the first mitotic division (63, 64). One of the first steps carried out at the male pronucleus is the replacement of protamines by histones. Due to the attachment of both ends of a DNA break to protamines and to the nuclear matrix, the DNA repair must happen at this phase, where two DNA ends remain tightly attached (62). In fact, DNA repair and paternal pronucleus replication mechanisms have been hypothesized to be linked to the nuclear matrix (60, 65–68). The results obtained here are similar to those obtained by Gawecka et al. (27) in a mouse model with induced double-stranded DNA breaks, where a delay was observed in the paternal pronucleus compared with the maternal pronucleus before the first embryo cleavage, in the presence of H2AX phosphorylation and chromosome aberrations (27). If DNA damage cannot be repaired during this short stage, embryo divisions can be performed but double-strand breaks may remain on the embryo, causing a split of chromosome fragments, chromosome reorganizations, or complex chromatin reorganizations to maintain chromosomal integrity (29, 48, 49, 69). These chromosomal aberrations may remain in some blastomeres and cause mosaicism, which is in fact an observation described in preimplantation genetic screening embryos (70–73). It is not until the morula stage that chromosome fragments can activate G1/S and G2/M checkpoints, triggering DNA damage apoptotic mechanisms on affected cells (29). In fact, when mouse sperm cells with induced dsSDF are used to fertilize p21 and p53 knockout embryos, where apoptosis is continuously suppressed even on day 3.5, embryos accumulate chromosomal aberrations and fail to implant (28). Relatedly, a higher implantation rate was observed when patients were classified as dsSDF (Table 1).

We finally selected the small subgroup of transferred embryos (n = 44) and analyzed the coincidence of embryo kinetics between embryos with and without successful implantation and low or high dsSDF groups (Tables 2 and 3). Embryos with successful implantation showed higher similarity to the low dsSDF group, whereas embryos without successful implantation showed higher similarity to the high dsSDF group. Therefore, embryos from the high dsSDF patients who achieved implantation might either have been successful on dsSDF repair or they come from a spermatozoon without dsSDF. Other studies have shown a delay of embryo kinetics at different stages to be related to lower implantation rates, and some investigators propose decision algorithms to select the best embryo to transfer (1, 3, 4, 74, 75). However, achieving a reduction in sperm double-strand break incidence could also be an important factor to improve implantation rates.

CONCLUSION

Double-stranded DNA damage, and not single-stranded DNA damage, has an effect in embryo kinetics and is related to implantation rates. The analysis of dsSDF in ICSI patients could be a relevant prognostic value for male-factor infertility in ICSI cycles.

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El daño del ADN espermático de cadena doble es causa de retraso en el desarrollo embrionario y puede afectar las tasas de implantación

Objetivo: Analizar el efecto de la fragmentación del ADN espermático de cadena simple y doble (ssSDF y dsSDF) en la cinética de embriones humanos monitorizada bajo un sistema de time-lapse.

Diseño: Estudio observacional, doble ciego, prospectivo de cohorte.

Entorno: Compañía derivada de una Universidad y centro privado.

Paciente(s): Se incluyeron prospectivamente ciento noventa y seis embriones de 43 parejas infértiles.

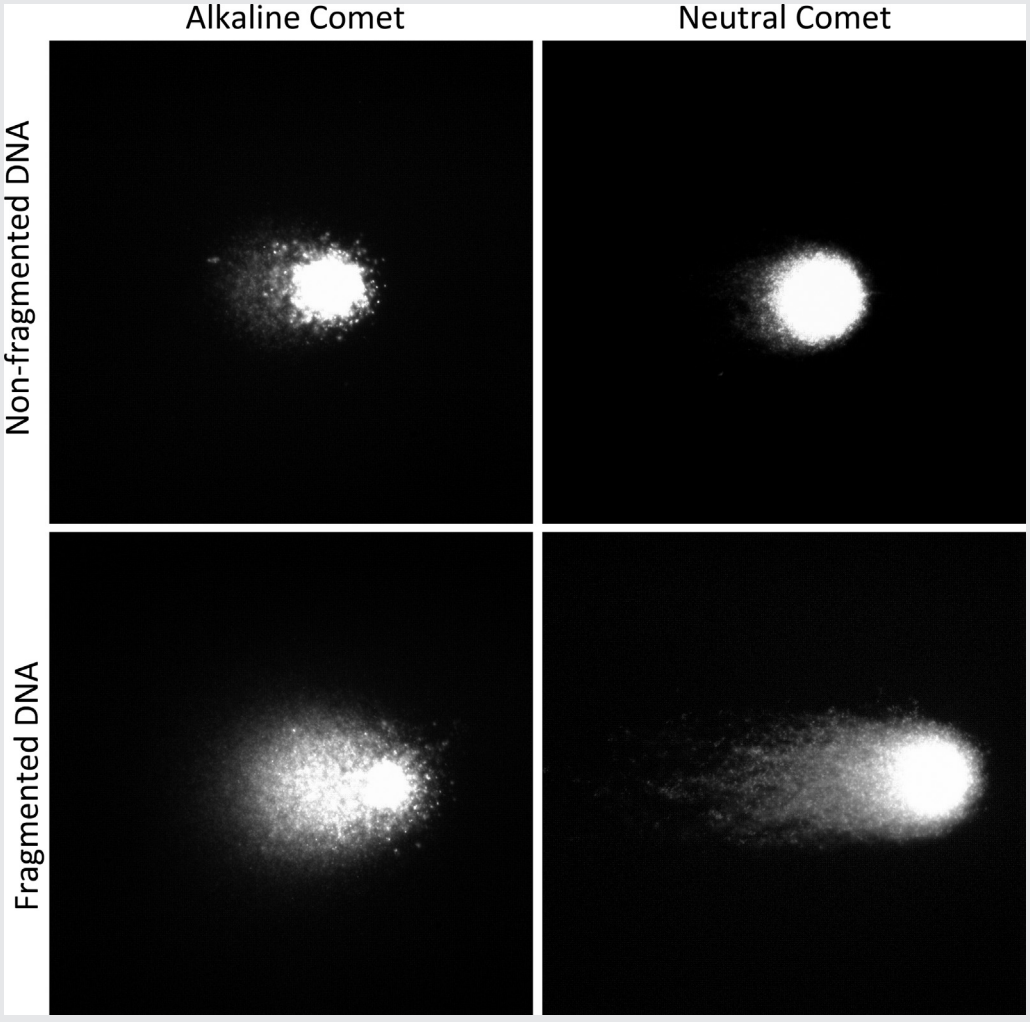
Intervención(es): Ninguna.

Principales medidas de resultado: ssSDF y dsSDF se analizaron en la misma muestra de semen utilizada para la inyección intracitoplasmática de espermatozoides. La cinética del embrión fue monitorizada utilizando tecnología time-lapse, y se obtuvo el tiempo de cada división del embrión.

Resultados: Cuando se comparan embriones obtenidos de muestras de semen con bajo dsSDF y alto dsSDF, al dividir los datos estadísticamente significativos el retraso en los de alto dsSDF se observó en la extrusión del segundo corpúsculo polar, T4, T8, mórula y las tasas de blastocistos temprano e implantación se vieron afectadas. La cinética de los embriones y las tasas de implantación no se ven significativamente afectadas cuando están presentes valores altos de ssSDF. Se observaron diferentes patrones de retraso en la cinética del embrión para estos diferentes tipos de daños en el ADN: dsSDF causó un retraso a lo largo de todas las etapas del desarrollo del embrión; sin embargo, su efecto principal se observó en las etapas de extrusión del segundo corpúsculo polar y mórula, coincidiendo en el punto de activación del daño del ADN embrionario descrito anteriormente; ssSDF tuvo su mayor efecto en la etapa de pronúcleos, pero la cinética del embrión se restauró en todas las etapas siguientes. Los resultados demuestran que dsSDF podría ser el principal tipo de daño en el ADN que afecta el desarrollo del embrión en los ciclos en que se realiza la inyección intracitoplasmática de espermatozoides, debido probablemente a que la selección espermática es basada en la movilidad en este procedimiento de reproducción asistida.

Conclusión: El daño en el ADN espermático de doble cadena causó un retraso en el desarrollo del embrión y una disminución en la implantación, mientras que el daño en el ADN de una sola cadena no afectó significativamente la cinética e implantación del embrión.

SUPPLEMENTAL FIGURE 1



Composition of nonfragmented and fragmented sperm cells visualized after alkaline or neutral Comet. Fragmented sperm cells show migrated DNA toward the cathode.

Casanovas. Double-strand breaks cause embryo delay. Fertil Steril 2018.