Original Article

Effect of Spermatic Nuclear Quality on Live Birth Rates in Intracytoplasmic Sperm Injection

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Abstract

Background: Our study defines the clinical role of sperm DNA damage in the assisted reproductive technology procedure. **Aim:** To investigate if the compaction of chromatin explored added to the analysis of the sperm DNA fragmentation allows obtaining a new indicator for sperm genome quality linked to live birth rate (LBR). **Design:** This was a prospective study, undergoing 101 cycles in the intracytoplasmic sperm injection (ICSI) program. **Materials and Methods:** The sperm DNA fragmentation index (DFI) has been measured with sperm chromatin dispersion examination. The sperm decondensation index (SDI) of chromatin has been measured with aniline blue procedure; with these indexes, a new parameter has been created: DFI × SDI. **Statistical Analysis:** Pearson's correlation coefficient, Student's *t*-test, and Chi-square test were used. The quantitative variables were described as mean ± standard deviation. Multivariate logistic regressions were performed with live birth as outcome. **Results:** The sperm concentration, motility, and normal morphology were lower when the DFI was high ($P = 0.001$). The fertilization rates and the number of obtained embryos were not statistically significant different according to the DFI groups. The SDI does not appear to be linked either with the spermatic parameters or with the ICSI parameters. A low DFI seems to be a beneficial factor to obtain a live birth in ICSI procedure ($P = 0.064$). In case of high DFI, a high SDI allows to obtain a higher LBR than a low SDI. **Conclusion:** The DFI is a good prognostic for a delivery rate in ICSI procedure, and the SDI could be added to DFI to create a new parameter of sperm nuclear quality. This new parameter seems to be linked to LBR.

Keywords: *Intracytoplasmic sperm injection, live birth rate, sperm chromatin condensation, sperm DNA fragmentation*

Introduction

The genome abnormalities are considered detrimental successful for the assisted reproductive technology (ART) procedure.[1] The intracytoplasmic sperm injection (ICSI) technique is an alternative to the problem of infertility, but the chance of live birth rate (LBR) is only about 25%.[2] Some of which may be related to the poor quality of the sperm genome.[3,4] For this reason, the analysis of the sperm genome before ART remains of paramount importance. Miscellaneous published studies indicate that the alteration of the spermatic genome leads to ART failures and demonstrate that the spermatozoa of infertile men have a much more altered sperm DNA than those of fertile men.[5-7] The sperm DNA plays a significant role in early embryogenesis development, subsequently, on the quality of the conceptus. A considerable number of the

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sperm DNA integrity investigations are proposed.[8-13] All of these procedures attempt to evaluate a potential relationship between the sperm DNA damage and embryo development and more generally the rate of the ongoing pregnancies.[14-17] The objective of the study was to investigate if the compaction of sperm DNA analyzed by the aniline blue procedure added to the examination of the sperm DNA fragmentation measured by SCD technique allows to obtain a new indicator for sperm DNA quality, new parameter linked to LBR.

Materials and Methods

Patients

The semen of males of a total of 101 couples undergoing an ICSI procedure from December 2014 to December 2015 was included in the study. Men with known pathologies involved in sperm DNA fragmentation were excluded such as cryptorchid testis, or varicocele or recent sperm infection, as well as testicular or epididymal sperm.

Assisted reproductive technology procedure

The ovarian stimulation was achieved using antagonist protocol; the ICSI procedure was carried out as described by Palermo *et al*., 1992.[18] Embryos obtained were classified according to Fragouli *et al*., 2013.[19] Two or three embryos were transferred depending on the age of the women and mostly on the quality of the obtained embryos. A clinical pregnancy was confirmed by increasing plasma beta-HCG concentration measured at three successive time points, followed by ultrasound detection of heartbeat; the LBR was used as the outcome of the ART procedure.

The semen was collected and analyzed according to the 2010 World Health Organization recommendations. The spermatozoa selection was performed with the procedure used routinely in our laboratory. A discontinuous gradient of PureSperm (PureSperm, Nicadon, Gothenburg, Sweden) constituted of two layers of PureSperm: one mL layer of PureSperm 90% and one mL layer of PureSperm 45% were used. One milliliter of sperm was placed on top of the 45% layer. After centrifugation (300 g for 20 min) at room temperature, the 90% layer was collected and washed with 2 mL of FertiCult flushing medium (FertiPro N.V., Beernem, Belgium) at 600 g for 10 min at room temperature. The pellet of sperm was resuspended in 200 µL of FertiCult IVF medium (FertiPro N. V). The semen was hold at 37°C until its use for ICSI procedure.

DNA fragmentation study by SCD technique

The SCD KIT Halosperm (Halotech DNA, Madrid, Spain) was used for sperm DNA fragmentation quantification according to the procedure described by Fernández *et al*., 2003.[8] Briefly, 50 µL of low-melting point agarose (Halotech DNA Kit, Madrid, Spain) at 0.65% was melted in a water bath at 90°C–100°C for 5 min and then set in an oven at 37°C for 5 min for temperature equilibration. Twenty-five microliters of density gradient sperm selected containing 5–10 million spermatozoa/mL were gently mixed with the agarose. Twenty microliters of the mixture were dropped on a slide. The dropped mixture was covered by an 18 mm × 18 mm coverslip and the slides were incubated at 4°C for 5 min. The slides were immersed in denaturation HCl solution (Halotech DNA Kit) for 7 min. A lysis step was performed during 20 min in dithiothreitol (Halotech DNA Kit)+ triton X-100 (Halotech DNA Kit) solution, and then, the slides were dehydrated in increasing concentrations of ethanol (70%, 90%, and 100%) (Sigma Aldrich Saint-Louis, MO, USA) for 2 min for each bath. The sperm cells were colored using eosin (Halotech DNA Kit) for 7 min and Azur blue (Halotech DNA Kit) for 7 min. Five hundred sperm cells were counted by patient to calculate the DNA fragmentation index (DFI).

Aniline blue cells sperm staining

The procedure has been originally described by Terquem and Dadoune, 1983.[13] Briefly, 20 µL of sperm selected with density gradient preparation was smeared on cleaned slides. The smeared sperm was fixed with formaldehyde 4% (Sigma Aldrich) for 5 min. The slides were subsequently washed for 5 min with 1X phosphate-buffered saline solution (%) (Sigma Aldrich) and dried. The nucleus sperm cells were stained 5 min in an aniline blue (Sigma Aldrich) solution at 5%, pH (2.5–3) with 4% acetic acid (Sigma Aldrich), in distilled water at room temperature. The slides were dehydrated in three baths of ethanol (70%, 96%, and 100%) for 1 min each one. The slides were subsequently immerged in two successive baths of methylcyclohexane solution (Sigma Aldrich) for 60 s each. In total, at least 200 sperm cells were examined for each patient and the sperm decondensation index (SDI) was determined by the number of spermatozoa with blue-stained head divided by the total number of spermatozoa count multiplied by 100.

Statistical analysis

The statistical analysis was conducted using SPSS software (SPSS 18.1, IBM, Chicago, IL, USA). Pearson's correlation coefficient, Student's *t*-test, and Chi-square test were used for univariate analysis. The variables were described as mean ± standard deviation (SD) for quantitative variables and with the distribution of percentage for categorical variables.

Multivariate logistic regressions were performed; in this case, the DFI and SDI parameters were used as a categorical variable. For DFI, the following subsets were defined ≤30% and >30%;[8] for SDI, the following subsets were defined <20% and ≥20%.[20] The DFI and SDI parameters were combined to create a new parameter to synthetize the sperm nucleus quality: DFI × SDI. The DFI × SDI parameter belonging three levels: level 1 was constituted with (DFI ≤30%), level 2 was constituted with (DFI >30% and SDI <20%), and level 3 was constituted with (DFI >30% and SDI ≥20%). A test was considered statistically significant when $P < 0.05$.

Results

Description of the population

The study involved 101 couples who underwent ICSI (corresponding to 101 cycles, one cycle per couple) with an average duration of sterility 7.6 ± 4.0 years. The average age of the men was of 38.9 ± 6.2 years [Table 1]. Oligoasthenozoospermia was present in 42.0% of the male population, oligozoospermia in 5.0%, asthenozoospermia in 25.0%, and normozoospermia in 28.0%. The average of sperm concentration was 17.3 ± 20.7 million/mL and the average sperm mobility was 27.3% ±21.6%. The percentage of spermatozoa with normal morphology was 79.4% ±14.5%. Concerning women factors, the average women's age was of 32.4 ± 4.4 years. The mean of ART rank was 1.4 ± 0.7. Among the included couples, 16.8% (17/101) have a miscarriage in their history. Among these miscarriages, 52.9% (9/17) were the outcome of an ART procedure and 47.1% (8/17) were the outcome of a natural pregnancy. In the present study, a total of 797 oocytes were retrieved, and the average was 7.9 ± 3.1, with an average of number of metaphase II oocytes of 6.0 ± 2.7. The fertilization rate (mean ± SD) was 82.1% ±20.9%. The mean number of embryos obtained per couple was 4.5 ± 2.4 from a total of 452 embryos. Among the obtained embryos, the rate of embryos Grade A quality was 51.2% ±33.8%. The mean number of transferred embryos was 2.3 ± 0.7 embryos per couple and the LBR was 20.8% [Table 1].

Sperm DNA fragmentation index and assisted reproductive technology procedure

No correlation was found between DFI and male age ($R = 0.06$, $P = 0.566$). Patients were divided into groups according to the DFI threshold value of 30%.[8] No statistically significant difference was found for male age (mean ± SD) according to the two groups of DFI: low (≤30%) and high DFI (>30%), 38.4 ± 5.4 years versus 39.1 ± 6.6 years with $P = 0.588$. The sperm concentration was lower when the DFI was high, 11.8 ± 17.6 million/mL versus 26.4 ± 22.4

Table 1: Cycle characteristics and assisted reproductive technology outcome according to DNA fragmentation index and sperm decondensation index (mean±standard deviation)

Patients' parameters	DFI		*P*	SDI		*P*	Total
	≤30%	>30%		<20%	≥20%		
Number of patient	*n*=38	*n*=63		*n*=27	*n*=61		*n*=101
Male age (years)	38.4±5.4	39.1±6.6	0.588	38.3±6.8	39.3±6.2	0.486	38.9±6.2
Sperm concentration (M/mL)	26.4±22.4	11.8±17.6	0.001	18.2±19.0	18.0±22.4	0.974	17.3±20.7
Sperm motility (%)	38.7±19.7	20.5±19.8	0.001	31.6±19.0	26.8±22.2	0.333	27.3±21.6
Sperm normal morphology (%)	83.5±14.8	77.1±13.8	0.046	75.7±17.5	81.1±12.7	0.112	79.4±14.5
Female age (years)	33.2±4.0	32.0±4.6	0.162	32.2±3.8	32.4±4.7	0.893	32.4±4.4
Sterility duration (years)	7.8±3.7	7.4±4.2	0.665	7.7±4.2	7.5±3.9	0.851	7.6±4.0
DFI (%)	-	-	-	40.4±20.1	47.3±23.2	0.159	44.4±21.9
SDI (%)	22.2±11.4	31.1±16.2	0.007	-	-	-	27.9±15.2
ART rank	1.4±0.7	1.4±0.7	0.926	1.4±0.8	1.4±0.7	0.841	1.4±0.7
Oocyte retrieved (*n*)	7.3±2.7	8.3±3.2	0.126	7.6±3.2	7.9±3.1	0.664	7.9±3.1
MII oocytes (*n*)	5.4±2.2	6.4±2.9	0.077	6.0±2.8	6.0±2.6	0.984	6.0±2.7
Fertilization rate (%)	86.4±20.3	79.5±21.0	0.111	87.5±22.0	80.2±20.6	0.134	82.1±20.9
Embryo obtained (*n*)	4.3±1.6	4.7±2.7	0.530	4.6±2.3	4.5±2.4	0.781	4.5±2.4
Grade A (%)	46.4±32.7	54.2±34.4	0.267	49.0±30.2	50.7±34.3	0.828	51.2±33.8
Grade B (%)	35.4±26.8	31.8±29.8	0.551	35±25.3	34.8±30.2	0.978	33.2±28.6
Grade C (%)	9.2±15.6	13.1±24.6	0.389	14.7±23.3	8.3±17.7	0.146	11.6±21.7
Grade D (%)	3.7±16.8	2.0±8.5	0.503	1.7±6.4	2.7±13.5	0.169	2.7±12.2
Transferred embryos (*n*)	2.4±0.7	2.3±0.6	0.158	2.4±0.7	2.3±0.7	0.493	2.3±0.7
Live birth rate (%)	28.9	15.9	0.117	18.50	23.0	0.641	20.8

DFI=DNA fragmentation index, SDI=Sperm decondensation index, SD=Standard deviation, MII=Metaphase II, ART=Assisted reproductive technology

million/mL with $P = 0.001$. The percentage of motile spermatozoa was lower when the DFI was high, 20.5% ± 19.8% versus 38.7% ± 19.7% with $P = 0.001$. The percentage of normal morphology spermatozoa was lower when the DFI was high, 77.1% ± 13.8% versus 83.5% ± 14.8% with $P = 0.046$. The correlation coefficient between DFI and SDI was significant and equal to 0.340 ($P = 0.001$). The SDI was lower when the DFI was high, 22.2% ± 11.4% versus 31.1% ± 16.2%, with $P = 0.007$. The fertilization rates were not different according the DFI group (low DFI group vs. high DFI group), 86.4% ± 20.3% versus 79.5% ± 21.1% with $P = 0.111$. The number of obtained embryos was not statistically significant different according to the DFI groups (low DFI group vs. high DFI group), 4.3 ± 1.6 versus 4.7 ± 2.7 with $P = 0.530$. No relationship was shown between embryo quality (rate of Grade A embryo quality) and the DFI group (low DFI group vs. high DFI group), 46.2% ± 32.7% versus 54.2% ± 34.4% with $P = 0.267$. The LBR was 28.9% (DFI ≤30%) versus 15.9% (DFI >30%), with not statistically significant difference ($P = 0.117$) [Table 1].

Sperm DNA decondensation index (SDI) and assisted reproductive technology procedure

Table 1 shows the main parameters in 88 patients for whom SDI has been quantified. Two groups were constituted according to SDI values:[20] Group 1 was constituted with SDI value <20% and Group 2 was constituted with SDI value ≥20%. No correlation was found between SDI values and male age ($R = 0.09$ with $P = 0.435$) and no statistically significant difference was found for male age (mean ± SD) according to the two SDI groups: 38.3 ± 6.8 years (Group 1) versus 39.3 ± 6.2 years (Group 2) with $P = 0.486$. No statistical differences for semen parameters were found according to the SDI groups. The sperm concentration (mean ± SD) for SDI Group 1 versus ≥Group 2 was 18.2 ± 19.0 million/mL versus 18.0 ± 22.4 million/mL, with $P = 0.974$. The sperm motility was 31.6 ± 19.0% versus 26.8% ± 22.2%, with $P = 0.333$. The normal sperm morphology was 75.7% ± 17.5% versus 81.1% ± 12.72%, with $P = 0.112$. The DFI rate according to SDI groups was no significantly different, 40.0% ± 21.1% (Group 1) versus 47.3% ± 23.2% (Group 2) with $P = 0.159$. The fertilization rate was no significantly different: 87.5 ± 2.2% (Group 1) versus 80.2% ± 20.6% (Group 2), with $P = 0.134$. The mean number of obtained embryo obtained was no significantly different: 4.6 ± 2.3 (Group 1) versus 4.5 ± 2.4 (Group 2), with $P = 0.781$. No relationship has been shown between sperm chromatin condensation and embryo quality (rate of Grade A embryo quality): 49.0% ± 30.2% (Group 1) versus 50.7% ± 34.3% (Group 2) with $P = 0.828$. The LBR was no significantly different according the SDI group: 18.5% (Group 1) versus 23.5% (Group 2) with $P = 0.641$.

Prognosis factors for live birth

The averages of DFI or SDI were not different according the success or the failure of the ART procedure. The average of DFI was 45.1% ± 22.5% (live birth failure) versus 41.5% ± 19.4% (live birth success), with $P = 0.501$. The average of SDI was 27.9% ± 15.8% (live birth failure) versus 27.7% ± 13.0% (live birth success), with $P = 0.962$ [Table 2]. When the DFI was ≤30%, the LBR was maximum 28.9% (11/38); the LBR was minimum 12.5% (2/16) when DFI was >30% with a SDI <20% [Tables 3 and 4]. With the logistic regression, the DFI seems to be the only prognosis parameter for live birth (odd ratio [OR] = 0.304, with $P = 0.064$) [Table 5]. The logistic regression performed with DFI × SDI parameters has shown that the level 1 (DFI ≤30%) provided the best results in terms of live birth even if it remains statistically no significant, and the chance of live birth was lower with level

Table 2: Cycle characteristics according to live birth (mean±standard deviation)

Patients' parameters	Live birth		*P*
	No	Yes	
Number of patient	*n*=80	*n*=21	
Male age (years)	39.2±6.5	37.4±4.7	0.221
Sperm concentration (M/mL)	16.3±20.9	21.0±20.1	0.358
Sperm motility (%)	26.2±21.9	31.5±20.3	0.321
Sperm normal morphology (%)	80.4±13.1	75.7±18.7	0.312
DFI (%)	45.1±22.5	41.5±19.4	0.501
SDI (%)	27.9±15.8	27.7±13.0	0.962
Female age (years)	32.5±4.4	32.1±4.6	0.725
Sterility duration (years)	7.5±4.2	7.8±3.3	0.756
ART rank	1.3±0.6	1.7±1.0	0.080
Oocyte retrieved (*n*)	7.8±3.1	8.3±2.8	0.510
MII oocytes (*n*)	6.0±2.8	6.1±2.2	0.829
Fertilization rate (%)	81.1±21.8	85.8±16.8	0.357
Embryo obtained (*n*)	4.4±2.4	5.0±2.1	0.316
Transferred embryos (*n*)	2.3±0.7	2.4±0.5	0.677

DFI=DNA fragmentation index, SDI=Sperm decondensation index, SD=Standard deviation, MII=Metaphase II, ART=Assisted reproductive technology

Table 3: Live birth rate according to sperm decondensation index and DNA fragmentation index as categorical parameters

Rates	DFI ≤30%	DFI >30%	Total
SDI <20%	27.3% (3/11)	12.5% (2/16)	18.5% (5/27)
SDI ≥20%	33.3% (7/21)	17.5% (7/40)	23.0% (14/61)
Total	31.3% (10/32)	16.1% (9/56)	21.6% (19/88)

DFI=DNA fragmentation index, SDI=Sperm decondensation index

Table 4: Assisted reproductive technology characteristics and outcome according to DNA fragmentation index × sperm decondensation index as categorical parameters

Patients' parameters	DFI ≤30%	DFI >30% and SDI <20%	DFI >30% and SDI ≥20%	*P*
Number of patient	*n*=38	*n*=16	*n*=40	
Male age (years)	38.4±5.4	38.1±7.7	39.8±6.5	0.509
Sperm concentration (M/mL)	26.4.7±22.4	13.2±15.2	12.4±19.6	0.007
Sperm motility (%)	38.7±19.7	27.9±20.9	19.8±19.4	0.001
Sperm normal morphology	83.5±14.8	73.4±14.5	78.6±13.5	0.065
Fertilization rate (%)	86.4±20.3	81.5±25.4	78.1±19.9	0.229
Embryo obtained (*n*)	4.3±1.6	4.4±2.9	4.9±2.8	0.623
Grade A (%)	46.4±32.7	41.8±27.8	57.9±34.3	0.158
Grade B (%)	35.4±26.8	39.4±27.9	32.6±30.7	0.720
Grade C (%)	9.5±15.8	18.3±27.6	8.3±17.7	0.211
Grade D (%)	3.8±17.0	1.8±7.3	1.3±5.8	0.616
Transferred embryos (*n*)	2.4±0.7	2.2±0.5	2.3±0.7	0.394
Live birth rate (%)	28.9 (11/38)	12.5 (2/16)	17.5 (7/40)	0.299

DFI=DNA fragmentation index, SDI=Sperm decondensation index, SD=Standard deviation

Table 5: Logistic regression with live birth as outcome

Features	Multivariate logistic regression		
	OR	95% interval of confidence	*P*
SDI ≥20%	1.646	0.458-5.914	0.445
DFI >30%	0.304	0.086-1.072	0.064
Female age (years)			
<30	1.000	-	-
30-35	0.479	0.107-2.147	0.336
36-40	0.973	0184-5.155	0.974
≥40	0.352	0.024-5.165	0.446
Oocytes retrieved (*n*)	1.109	0.891-1.381	0.353
Fertilization rate (%)	1.014	0.983-1.046	0.391
Embryo transferred (*n*)	1.180	0.522-2.667	0.691

DFI=DNA fragmentation index, SDI=Sperm decondensation index, OR=Odds ratio

2 (DFI >30% and SDI <20%), OR = 0.334 (*P* = 0.205) and with level 3 (DFI >30% and SDI ≥20%), OR = 0.428 (*P* = 0.105) [Table 6].

Discussion

Our results showed no relationship between sperm DNA damage and men age and no difference was found for male age according to the two groups of DFI. The relationship between DFI and age of men was already proven in other studies.[21,22] The absence of relationship is probably a consequence of the small size of our cohort compared with these two studies. The study of the relationship between semen parameters and DFI has shown that the alteration of spermatic parameters such as the concentration, the motility, and the morphology was inversely associated with the DFI. These results confirmed those found by other authors.[1,14,23-25] Any association between DFI and a failure of fertilization was observed; this result concords with what has been already described[15,16,26-29] and discords with some other studies.[30,31] This result indicates that sperm with high DNA damages can undergo successful fertilization, pronuclear formation, and syngamy as it was previously described.[27] Høst *et al*., 2000[32] assumed that in ICSI, an embryologist tries to select a motile, and as possible, some morphologically normal spermatozoa, so it can be hypothesized that spermatozoa with low DFI are used for an ICSI procedure. However, it can be argued that a spermatozoon can be considered as "normal" and at the same time has impaired DNA.[33] Moreover, with ICSI, the barriers of natural selection are bypassed and can possibly be fertilized with highly fragmented DNA sperm, as it was found that the oocyte can repair the damaged DNA.[34-36] This information could explain that the comparison of the amount of embryo obtained between two groups of DFI (low DFI and high DFI groups) shows that there is no influence of the sperm DNA fragmentation on embryo development.[30,37,38] Zini *et al*., 2011[39] have supported the fact that an excessive damage can potentially lead to failures at the quality level or development of the embryo. In our survey, the quality of the embryos in the early stages of the development does not seem to be affected by the quality of the spermatic genome. No relationship has been shown between embryo quality and high DNA fragmentation; our results agree to what has already been observed.[14,26,40] The first stages of embryo development depend on maternal transcripts and that the paternal influence only begins at the six to eight cells stage, which explains the absence a relationship between DFI and embryo development until day 3. In our study, embryo transfers were performed on day 2 or 3 after follicles retrieval; before the paternal influence would be felt,[41] we found a positive correlation between

Table 6: Logistic regression with combined DNA fragmentation index × sperm decondensation index and with live birth as outcome

Features	Multivariate logistic regression		
	OR	95% interval of confidence	*P*
DFI × SDI			
DFI ≤30%	1.000	-	-
DFI >30% and SDI <20%	0.334	0.061-1.817	0.205
DFI >30% and SDI ≥20%	0.428	0.131-1.401	0.161
Female age (years)			
<30	1.000	-	-
30-35	0.398	0.089-1.779	0.371
36-40	0.781	0.168-3.638	0.962
≥40	0.465	0.033-6.529	0.570
Oocytes retrieved (*n*)	1.072	0.881-1.303	0.489
Fertilization rate (%)	1.012	0.983-1.041	0.437
Embryo transferred (*n*)	1.097	0.434-2.774	0.844

DFI=DNA fragmentation index, SDI=Sperm decondensation index, OR=Odds ratio

DFI and SDI. The relationship between sperm DNA fragmentation and sperm chromatin compaction leads us to hypothesize that DNA damage could be related to protamine content as it was stated by some studies.[42,43] We could hypothesized that a defect during the sperm protamination could lead to an increase of fragmented sperm DNA. Concerning the SDI results, no difference was noted between the male ages according to the two SDI groups. This result is in agreement with Belloc e*t al*., 2009,[21] who found the same result. No statistical relationship with SDI and sperm parameter studied whether for concentration, motility, or morphology, as it was previously described.[44-46] No relationship was found between SDI and fertilization rate or between SDI and early embryonic development. These results confirm the study performed by Hammadeh *et al*., 1996.[47] As SDI belongs to paternal factors of embryo development, it seems realistic that SDI is weakly involved during the early embryo development. This study reported also that abnormal packaging of the sperm chromatin has no impact on the quality of the embryo, as described by Sadeghi *et al*., 2009.[48] Regarding the LBR, a high DFI value decrease the chance of live birth as it was previously stated.[1,2,12,14,16,49] Furthermore, the higher LBR was obtained within a case of sperm with low DFI value independently of SDI values. The paradoxical result was that the lowest LBR was obtained in case of sperm with high DFI value and low SDI value and not in case of high DFI value and high SDI value. We could hypothesize that the high DFI and high SDI originated from the same genetic failure, and this failure could be repaired by the oocyte DNA repair toolkits. In case of high DFI and low or normal SDI, the failure was originated by another genetic mechanism and this failure will be more difficult to be repaired by the oocyte DNA repair toolkits, which result in a low LBR. The SDI could be added to DFI to create a new parameter of sperm nuclear quality. In case of high DFI value, SDI could allow the identification of a good prognostic group for live birth: high DFI value and high SDI value. However, a spermatozoa with good quality of DNA (low DFI) can induce a live birth even if its chromatin is poorly compacted (high SDI) and when the spermatozoa has an altered DNA (high DFI) but has a good packing quality (low SDI), the possibility of births would be low.

Our study has some limits. The main limit is that no classical IVF procedure has been included. In fact, all our patients were oriented by their doctors to the ICSI procedure and the choice of the patient themselves; subsequently, the medical settlement is at their charge. Their choice is therefore dictated by financial reasons for maximizing their chances of a pregnancy. No transfer at blastocyst stage was performed, so the relationship between DFI, SDI, and DFI × SDI could not be studied.

Conclusion

This prospective study confirms the relationship between the DFI and LBR. In addition, the prognostic value of DFI could be increased if the SDI is quantified at the same time. A poor prognostic group has been identified: high DFI and low SDI. The next step will be to identify treatments able to decrease sperm DFI and/or increase sperm SDI to improve the ART results.

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Conflicts of interest

There are no conflicts of interest.

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