ABSTRACT

Background and Objective: Ongoing debates exist regarding the impact of sperm DNA fragmentation (SDF) on clinical IVF outcomes. The aim of this study is to investigate whether SDF levels affect fertilization rate, pregnancy rate and miscarriage rate in couples undergoing fresh IVF cycles.

Method: This retrospective study investigated 169 consecutive fresh IVF cycles performed between January 2012 and June 2014. Semen was collected on the day of oocyte retrieval and standard sperm quality was assessed in the raw semen. In addition to standard semen parameters, SDF was also measured by TUNEL method. Poor ovarian responder (POR) was defined when three or less mature oocytes were collected. Oocytes were inseminated by the conventional method (n = 69) or by ICSI (n = 95) depending on the quality of sperm and oocyte. The embryos were transferred three or five days after oocyte retrieval. Miscarriage was defined as pregnancy loss before 12 weeks of gestation.

Results: SDF level did not affect fertilization rate or pregnancy rate, but SDF level did have a significant effect on miscarriage rate. In the miscarriage group (n = 10), SDF level was significantly higher (23.9% vs. 14.1%) and number of mature oocytes was significantly lower (4.3 vs. 7.6) when compared with the live birth group (n = 45). Multiple regression analysis showed that SDF level was an independent predictor for miscarriage (OR 1.051, 95% CI 1.001 - 1.104).

For prediction of miscarriage, the cutoff for SDF level was >13% and the cut-off for number of mature oocytes was ≤3. In the low SDF group (≤13%), miscarriage rate was similar between POR and normal ovarian responder (NOR) (14.2% vs. 4.3%). In the high SDF group (>13%), miscarriage rate was significantly higher in POR than NOR (60% vs. 13.3%, p = 0.045).

Conclusion: This study demonstrated that high SDF level (>13%) was associated with high miscarriage rate. High SDF level contributes to miscarriage only in the POR group. The results suggest that SDF measurement should be considered in couples with POR in order to predict the prognosis of the IVF pregnancy.

Keywords; sperm, DNA fragmentation, in vitro fertilization, pregnancy, miscarriage

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LIST OF TABLES

LIST OF FIGURES

Introduction

Although sperm DNA fragmentation (SDF) is not routinely assessed in the semen analysis, it can be offered as a special test.[1] There are still ongoing debates on whether SDF should become a part of the routine fertility workup.[2] There have been number of studies evaluating the influence of SDF on fertilization, embryo quality and pregnancy outcomes and five meta-analyses are currently provided (Table 1).[3-7]

Considering high quality evidences from the meta-analyses, high SDF level appears to be associated with lower rate of clinical pregnancy in standard IVF cycles, but not with pregnancy in intracytoplasmic sperm injection (ICSI) cycles.[3,5,6] Significantly lower live birth rate in high SDF group was reported and this negative impact was prominent in IVF cycles.[7] Moreover, high SDF level appears to be associated with higher miscarriage rate in ICSI cycles only[5] or in overall cycles[4], although some studies reported no significant association with miscarriage rates.[6] Regarding fertilization rate, one meta-analysis reported no association with SDF level in both standard IVF and ICSI cycles.[3]

Although positive associations between SDF level and several IVF outcomes have been reported, the majority of the studies have not reported a clear cut-off value of SDF level for the prediction of poor fertilization rate, clinical pregnancy, live birth or miscarriage.

Table 1. Meta-analyses investigating effect of sperm DNA fragmentation on IVF outcomes

Study	Results
Li et al. (2006)	·No association with fertilization rate in both IVF/ICSI cycles ·Lower clinical pregnancy rate in high SDF group in IVF cycles (RR 0.68, CI 0.54 - 0.85, p = 0.006), but not in ICSI cycles
Robinson et al. (2012)	·Significant increase in miscarriage in high SDF group compared with low SDF group (RR 2.16, CI 1.54 - 3.03, p <0.005)
Zhao et al. (2014)	·Lower clinical pregnancy rate in high SDF group in IVF cycles (RR 0.66, CI 0.48 - 0.90, p = 0.008), but not in ICSI cycles ·Higher miscarriage rate in only ICSI cycles (OR 2.68, CI 1.40 - 5.14, p = 0.003)
Zhang et al. (2015)	·No association between SDF level and clinical pregnancy rate or miscarriage rate
Osman et al. (2015)	·Significant increase in live birth rate in low SDF group compared with high SDF group (RR 1.17, CI 1.07 - 1.28, p = 0.0005)

When investigating the association of SDF level with fertilization rate, previous studies have divided the cycles into standard IVF group and ICSI group. However, since indications for ICSI include not only male factors but also certain female factors, it is reasonable that ICSI groups be divided into male and female factor groups and evaluated separately.

In this study, we investigated the association between SDF level and several IVF outcomes such as fertilization rate, pregnancy rate and miscarriage rate. Whether SDF level was higher in couples with repeated IVF failure (RIF) was also assessed. When the association between SDF level and fertilization rate was evaluated, separate analyses were performed for standard IVF group, ICSI group due to male factor, and ICSI group due to female factors, respectively.

Materials and Methods

1. Study population

The dataset for this retrospective study included 169 consecutive fresh IVF cycles performed between January 2012 and June 2014 at the Seoul National University Bundang Hospital. This study was approved by the Institutional Review Board of the Seoul National University Bundang Hospital (IRB No. B-1608-357-108). The indications for IVF were unexplained infertility (n = 78), tubal factor (n = 26), age factor (n = 20), endometriosis (n = 18), uterine factor (n = 10), polycystic ovary syndrome (n = 9), and male factor infertility (n = 8). The body mass index, basal serum level of FSH, and random serum level of anti-Müllerian hormone (AMH) in female partners were recorded if they were measured within two months before starting the cycle.

2. Stimulation protocols and oocyte collection

Controlled ovarian stimulation was performed using recombinant FSH (Gonal-F, Serono, Geneva, Switzerland) with or without highly purified urinary gonadotropin (Menopur, Ferring, Malmo, Sweden) using the luteal long protocol of GnRH agonist (Decapeptyl 0.1 mg/d; Ferring) (n = 10) or the GnRH antagonist protocol (Cetrotide 0.25 mg/d; Serono) (n = 157). When two or more leading follicles reached a mean diameter of ≥18 mm, 250 μg of recombinant human chorionic gonadotropin (hCG) (Ovidrel, Serono) was injected. Oocytes were retrieved 36 hours after the hCG injection. Poor ovarian responder (POR) was defined if three or less mature oocytes were collected. MI-derived in vitro matured oocytes were counted as mature oocytes, but GV-derived oocytes were excluded.

3. Semen collection, measurement of SDF, and in vitro fertilization

Semen was collected on the day of oocyte retrieval and standard sperm quality was assessed in the raw semen (concentration, motility, and normal form by strict criteria). Sperm quality was defined as 'normal' (n = 104) when the semen parameters were within the WHO reference values regardless of patient diagnosis. 'Subnormal' group (n = 43) represents semen with parameters outside the WHO criteria, but not indicative of ICSI; 'abnormal' group (n = 22) were those who required ICSI.

SDF was measured by TUNEL method in the raw semen, as previously reported in our center.[8] Semen samples were smeared on a silane-coated slide (DAKO) and air dried. Samples were fixed with 4% paraformaldehyde for 1 hour at 15°C - 25°C, then washed with phosphate-buffered saline (PBS), and then were permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate (Sigma-Aldrich). A commercial apoptosis detection kit (In Situ Cell Death Detection Kit; Roche Diagnostics GmbH) was used to assess cell death in the samples according to the manufacturer's instructions. Counterstaining was performed using a mounting medium with 4',6-diamidino-2-phenylindole (DAPI; Vector Laboratories). The nuclei of sperm with fragmented DNA stained green, whereas the nuclei of other cells stained blue (Figure 1). Sperm heads with >50% of the area stained green were considered positive for DNA fragmentation. At least 500 sperms were counted per experimental set and SDF level was determined as the percentage of sperm with fragmented DNA.

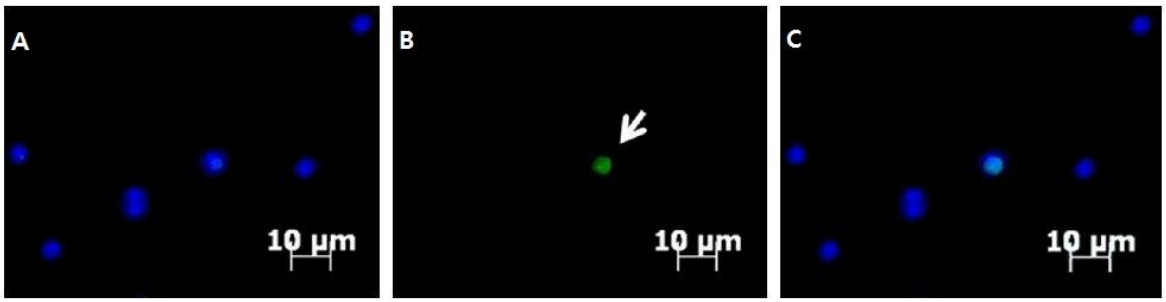

Figure 1. Representative microphotographs showing ejaculated sperm stained by 4',6-diaminidino-2-phenylindole (DAPI) (A, X400); green-colored sperm head (thick arrow) stained by TUNEL (B, X400); merged (C, X400).

The remained semen was processed by discontinuous gradient as described in the kit instruction (Sydney IVF density gradient media; COOK, Brisbane, Queensland, Australia). After initial centrifugation of the semen (300 X g for 5 minutes) for removal of the seminal plasma, obtained pellet was suspended in fresh Ham's F10 medium (1.5 mL) supplemented with 10% SSS (Irvine Scientific, USA). Pre-washed semen (1.0 mL) was layered on the top of a discontinuous gradient in a 15-mL conical tube (40%/80%). The conical tube was centrifuged at 300 X g for 5 min and sperms collected from the bottom layer (80% layer) were washed twice by re-suspension in 4 mL of Ham's F10 medium and centrifugation (300 X g for 5 min). After twice centrifugations, the supernatant was removed and the pellet re-suspended in 3 mL of Ham's F10 medium supplemented with 10% SSS. Re-suspended pellet was later used in insemination.

The oocytes were inseminated by the conventional method (n = 69) or by ICSI (n = 95) or by split insemination (n = 3), depending on the quality of the sperm and oocyte. ICSI was used in 22 cycles due to male factor and in 73 cycles due to female factors. Fertilization was confirmed by observing two-pronuclear zygote (2PN) on the day after oocyte retrieval. 'Low fertilization rate' in the current study was defined as ≤75%, which corresponded to the 25 percentile of the overall fertilization rate.

4. Embryo transfer and confirmation of pregnancy

The embryos were transferred three or five days after oocyte retrieval. Embryo quality was evaluated by morphological criteria based on degree of fragmentation and regularity of blastomeres on day 3 after fertilization. The embryos were graded as follows: grade A, 0% anucleate fragments, regularity of blastomeres, and no apparent morphologic abnormality; grade

B, <20% anucleate fragments, regularity of blastomeres, and no apparent morphologic abnormalities; grade C, 20-50% anucleate fragments, irregularity of blastomeres, and no apparent morphologic abnormality; and grade D, >50% anucleate fragments, irregularity of blastomeres, and apparent morphologic abnormalities. Blastocysts were evaluated on day 5 by development stage and quality of the inner cell mass and trophectoderm. A good quality blastocyst was defined as grade AA, AB, AC, BA, BB, or CA. Luteal phase support was performed using either a daily dose of 50 mg of P in oil (Progest, Genifer, Seoul, Korea) or 8% P gel (Crinone, Serono), starting on the day of oocyte retrieval. Pregnancy was first assessed 14 days after oocyte retrieval by measuring serum hCG level. In cases with positive hCG results, transvaginal ultrasonography was performed to confirm intrauterine pregnancy and to identify the number of gestational sacs and fetal heartbeat. Clinical pregnancy was defined as presence of one or more gestational sacs. Miscarriage was defined as pregnancy loss before 12 weeks of gestation.

5. Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences software (PASW ver. 18, SPSS Inc., Chicago, IL, USA). When analyzing the association between standard sperm quality and SDF level, the data from 169 sperm samples were used. When fertilization rates were analyzed, the data from 164 cycles were used (after excluding three cases with split insemination and two cases with no mature oocyte). Pregnancy rates were analyzed in 157 cycles in which embryo transfer was done, and miscarriage rates were analyzed in 55 cases which achieved clinical pregnancy. The correlation test was performed by non-parametric Spearman's rank test. The chi-squared test was used to compare proportions between two

groups. If the cell numbers were <5, Fisher's exact test was applied to compare frequencies between groups. The median of numeric data were compared using the Mann-Whitney test. Multiple regression analyses were performed for several numeric variables. A receiver operator characteristics (ROC) curve analysis was used to assess specific cut-off value for several numeric parameters. The result was considered significant when the p-value was <0.05 (two-tailed).

Results

1. SDF levels and standard sperm parameters

Level of SDF ranged from 0.4% - 56.8% in 169 sperm samples (mean ± standard deviation: 15.1% ± 12.2%). They did not show a normal distribution and the median value was 11.8%).

As shown in Table 2, in overall population, positive correlation between SDF and age of male was found but an inverse correlation was found between SDF and sperm motility. A multivariate analysis revealed that both male age and motility were significant variables (SDF = -0.444 + [0.618 x male age] - [0.162 x motility]). The positive correlation between SDF and male age was prominent in group with normal sperm, and the inverse correlation between SDF and motility was prominent in group with subnormal sperm. In group with abnormal sperm, no significant correlations were found between SDF and any of the standard sperm parameters.

In group with normal sperm, the median SDF level (95% confidence interval) was 11.0% (8.5% - 13%). The median SDF level was 13.0% (7.2% - 18.7%) in group with subnormal sperm and 14.9% (10.4% - 29.8%) in group with abnormal sperm. There was a significant difference between the median SDF level between normal sperm and abnormal sperm group ($p < 0.05$).

Table 2. Correlation coefficients to show an association between sperm DNA fragmentation with standard sperm parameters

	Overall (n = 169)	Sperm quality		
		Normal (n = 104)	Subnormal (n = 43)	Abnormal (n = 22)
Age of husband (years)	0.29†	0.31†	0.20	0.38
Volume (mL)	-0.14	-0.09	0.04	-0.25
Concentration (million/mL)	0.06	0.01	0.33†	0.27
Motility (%)	-0.21†	-0.06	-0.53†	-0.31
Total motile sperm (million)	-0.13	-0.08	-0.09	-0.14
Normal form (%)	0.07	0.01	0.22	-0.02

†$p < 0.05$ by Spearman's rank test.

2. SDF levels in couples with RIF

We defined RIF as failure to become pregnant after three or more previous IVF cycles. As shown in Table 3, 29 couples belonged to RIF group, and the significant factors were male and female age and SDF level.

Since male age was closely associated with SDF level as shown in Table 2, we stratified out data by male age (Table 4). In couples whose male partner was 42 years old or more, SDF level was not different between the RIF and the non-RIF group. However, SDF level was significantly higher in the RIF group, when male partner was 41 years old or less.

Table 3. Clinical characteristics in couples with repeated IVF failure (RIF)

	Non-RIF (n = 140)	RIF (n = 29)	p
Age of husband (years)	37.8 [37.1 – 38.6]	42.0 [39.0 – 43.8]	0.01
Sperm parameter			
Volume (mL)	3.1 [2.9 – 3.3]	2.5 [2.1 – 3.0]	NS
Concentration (million/mL)	145 [122 – 169]	88 [72 – 100]	NS
Motility (%)	51.2 [48.2 – 54.2]	51.0 [38.2 – 60.8]	NS
Total motile sperm (million)	203 [172 – 233]	96 [57 – 151]	NS
Normal form (%)	9.8 [8.8 – 10.8]	7.5 [4.7 – 9.8]	NS
Sperm DNA fragmentation (%)	14.4 [12.4 – 16.4]	17.2 [7.6 – 24.6]	0.03
Age of female (years)	35.4 [34.7 – 36.1]	39.0 [36.2 – 41.8]	0.01
Weight (kg)	56.9 [55.7 – 58.5]	58.3 [55.1 – 59.8]	NS
Height (cm)	160.5 [159.6 – 161.4]	160.0 [157.0 – 164.4]	NS
Serum level of FSH (mIU/mL)	6.2 [5.7 – 6.8]	6.3 [4.0 – 9.1]	NS
Serum level of AMH (ng/mL)	2.8 [2.3 – 3.3]	1.8 [0.8 – 3.2]	NS
Indication of IVF			0.001
Unexplained	71	7	
Tubal	22	4	
Age	11	9	
Endometriosis	16	2	
Uterine	5	5	
Polycystic ovary syndrome	8	1	
Male factor	7	1	

RIF: failure to become pregnant after three or more previous IVF cycles.

Median [95% CI].

Mann-Whitney U test.

Table 4. Sperm DNA fragmentation level in couples with repeated IVF failure (RIF) (stratified by age of husband)

	Age of husband ≤41 years (n = 125)			Age of husband ≥42 years (n = 44)		
	Non-RIF (n = 111)	RIF (n = 14)	P	Non-RIF (n = 29)	RIF (n = 15)	p
Sperm DNA fragmentation (%)	12.6 [10.6 – 14.5]	21.0 [13.2 – 28.9]	0.015	21.3 [15.4 – 27.2]	16.4 [10.6 – 22.3]	NS
Age of women (years)	34.4 [33.8 – 35.1]	36.3 [34.5 – 38.1]	0.022	39.1 [37.4 – 40.7]	41.3 [39.5 – 43.0]	NS

RIF: failure to become pregnant after three or more previous IVF cycles.

Median [95% CI].

Mann-Whitney U test.

3. Impact of SDF on fertilization rate

SDF level did not correlate with fertilization rate in overall population or in subgroups according to the insemination method or according to female or male factors (Figure 2). In three subgroups according to sperm quality, no significant correlation was found between SDF and fertilization rate in overall population, standard IVF group or ICSI group due to female or male factor (Table 5).

A ROC curve analysis was done to evaluate whether SDF levels may predict low fertilization rate (i.e. ≤75%). The cutoff value for SDF was ≤5.1% in the overall population, ≤4.9% in standard IVF group, >21.3% in ICSI group, >21.3% in ICSI group due to female factor, and >12.9% in ICSI group due to male factor. However, none of the values were statistically significant.

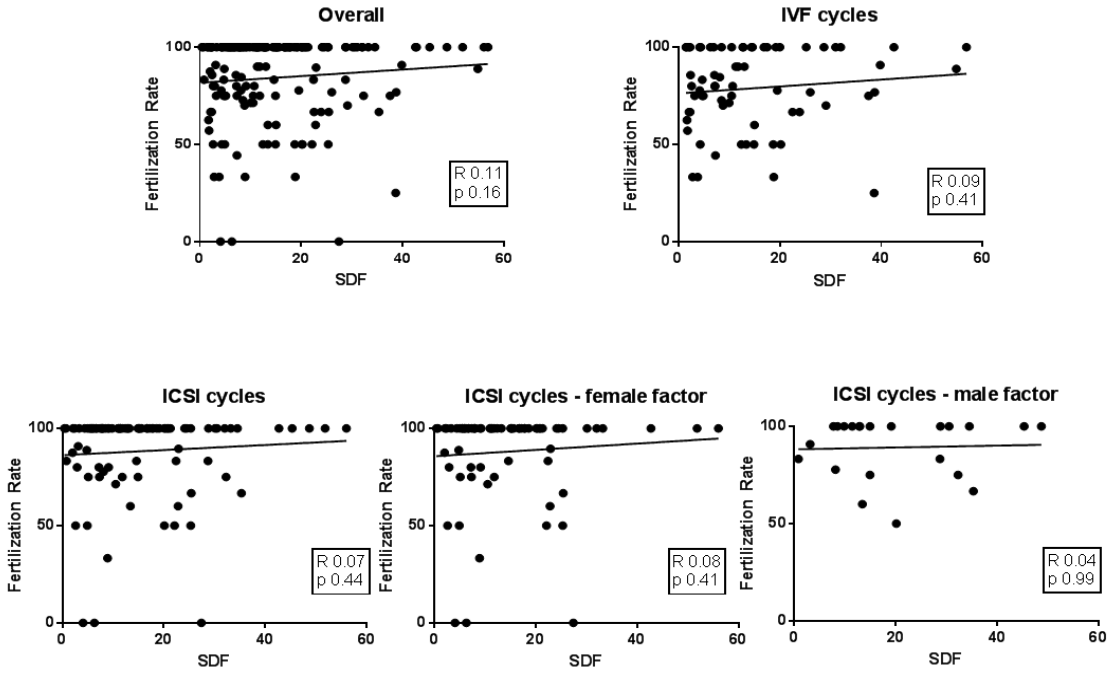

Figure 2. Association between sperm DNA fragmentation level and fertilization rate (FR) in overall study group or in subgroups

Table 5. Association between sperm DNA fragmentation level and fertilization rate (FR) in sub-groups according to sperm quality

	Sperm quality								
	Normal			Subnormal			Abnormal		
	FR	n	r	FR	n	r	FR	n	r
Overall	100%	102	-0.02	87.3%	40	0.28	100%	22	0.04
IVF group	84%	50	0.05	75%	19	0.27			
ICSI group (female factor)	100%	52	-0.09	100%	21	0.27			
ICSI group (male factor)							100%	22	0.04

FR is expressed as median value.

†p <0.05 by Spearman's rank test.

4. Impact of SDF on clinical pregnancy

Clinical pregnancy rate was 31% (40/129) in day 3 transfer and 53.5% (15/28) in day 5 transfer. Between pregnant and non-pregnant group, SDF levels and other standard sperm parameters did not differ (Table 6). Three factors showing significant difference between pregnant and non-pregnant group were identified; age of female in day 3 transfer group, age of husband and fertilization rate in day 5 transfer group.

5. Impact of SDF on miscarriage

Clinical pregnancy was achieved in 55 women, but 10 women ended in spontaneous miscarriage. In the miscarriage group, SDF level and serum FSH level were significantly higher and number of mature oocyte was significantly lower when compared with the live birth group (Table 7).

A ROC curve analysis revealed that each cutoff value for prediction of miscarriage was SDF level >13%, basal serum level of FSH >6.7 mIU/mL, and number of mature oocyte ≤3; all three cutoff values were statistically significant (Table 8, Figure 3).

Neither male nor female age was a predictor for miscarriage. Since both male and female age could act as confounders, multiple regression analysis was performed after including five parameters (SDF level, basal serum level of FSH, number of mature oocytes, male and female age); SDF was found to be the only significant factor for prediction of miscarriage (OR 1.051, 95% CI 1.001 - 1.104).

Table 6. Comparison of clinical and laboratory parameters between pregnant and non-pregnant women

	Day 3 transfer		Day 5 transfer	
	Pregnant (n = 40)	Not pregnant (n = 89)	Pregnant (n = 15)	Not pregnant (n = 13)
Age of husband (years)	37.5	39	34	38†
Volume (mL)	3	3	3	3
Concentration (million/mL)	97	97	73	138
Motility (%)	55.1	50.2	47	47.9
Total motile sperm (million)	151	129	106	128
Normal form (%)	8.5	9.4	10.7	7.6
Sperm DNA fragmentation (%)	13	12.4	8.8	9.2
Age of women (years)	35	37†	32	34
Cycle number	2	2	1	1
Serum level of FSH (mIU/mL)	6.6	6.3	4.7	4.8
Serum level of AMH (ng/mL)	1.8	1.6	4.1	3.5
Amount of gonadotropin (amp)	24	24	24	21
Peak serum level of estradiol (pg/mL)	1,133	847	2,533	2,647
Mature oocyte	4	3	11	10
conventional insemination	24	28	9	6
ICSI	16	59	5	6
split insemination	0	1	1	1
Fertilization rate (%)	84.5	89†	88.9	88.5
Embryo transferred	2	2	2	2
Grade A embryo*	1	1		
Grade A or B embryo*	2	2		
Endometrial thickness (mm)	8.8	8.4	10	8.7
Triple pattern endometrium	97.4%	82.8%	93.3%	92.3%

Expressed as median.

†$p < 0.05$ between pregnant and not pregnant group (Mann-Whitney U test).

*Day 3 transfer cycle only.

Table 7. Comparison between live birth group and miscarriage group

	Live birth (n = 45)	Miscarriage (n = 10)	p
Age of husband (years)	36.8 [35.4 – 38.3]	37.8 [34.6 – 41.1]	NS
Volume (mL)	3.1 [2.7 – 3.5]	3.2 [2.5 – 3.9]	NS
Concentration (million/mL)	133 [93 – 172]	135 [92 – 177]	NS
Motility (%)	51 [45.9 – 56]	55.9 [41.8 – 70]	NS
TMC (million)	184 [128 – 240]	214 [144 – 284]	NS
Normal form (%)	10.1 [8.2 – 11.9]	10.6 [6.2 – 15]	NS
Sperm DNA fragmentation (%)	14.1 [10.3 – 17.9]	23.9 [12.9 – 35]	0.038
Age of women (years)	33.9 [32.9 – 35]	36.1 [32 – 40.2]	NS
Cycle number	2 [1.6 – 2.5]	1.9 [0.7 – 3.1]	NS
Serum level of FSH (mIU/mL)	5.7 [4.9 – 6.5]	7.3 [5.9 – 8.8]	0.015
Serum level of AMH (ng/mL)	3.8 [2.7 – 4.8]	1.9 [0.6 – 3.2]	NS
Amount of gonadotropin (amp)	21.8 [20 – 23.7]	24.5 [20.1 – 28.9]	NS
Peak serum level of estradiol (pg/mL)	1,941 [1,450 – 2,431]	1,893 [732 – 3,053]	NS
No. of mature oocyte	7.6 [6 – 9.2]	4.3 [1.6 – 7]	0.025
conventional insemination	28	5	NS
ICSI	16	5	
split insemination	1	0	
Fertilization rate (%)	83 [77.3 – 88.7]	76.9 [58.9 – 95]	NS
Day 3 transfer	32	8	NS
Day 5 transfer	13	2	
Embryo transferred	2 [1.8 – 2.2]	1.6 [1.1 – 2.1]	NS
Grade A embryo*	1 [0.4 – 1.6]	1 [0.9 – 1.5]	NS
Grade A or B embryo*	2 [1.7 – 2.2]	1 [0.9 – 1.8]	NS
Endometrial thickness (mm)	8.8 [8.3 – 10]	10.6 [8 – 16]	NS
Triple pattern endometrium	95.5%	90%	NS

Median [95% CI].

Mann-Whitney U test.

*Day 3 transfer cycle only.

Table 8. Prediction of miscarriage by using five parameters

	Cutoff	AUC	95% CI	Sens	Spec	+LR	-LR	+PV	-PV
Sperm DNA fragmentation (%)	>13	0.713†	0.575 – 0.827	80.0	62.2	2.12	0.32	32.0	93.3
Serum level of FSH (mIU/mL)	>6.7	0.768†	0.614 – 0.883	77.8	79.4	3.78	0.28	50.0	93.1
Mature oocyte	≤3	0.733†	0.597 – 0.843	70.0	77.8	3.15	0.39	41.2	92.1
Age of husband (years)	>36	0.570	0.429 – 0.703	70	53.3	1.50	0.56	25.0	88.9
Age of women (years)	>35	0.622	0.481 – 0.749	60	73.3	2.25	0.55	33.3	89.2

†; $p < 0.05$.

Sens: sensitivity, Spec: specificity, +LR: positive likelihood ratio, -LR: negative likelihood ratio, +PV: positive predictive value, -PV: negative predictive value.

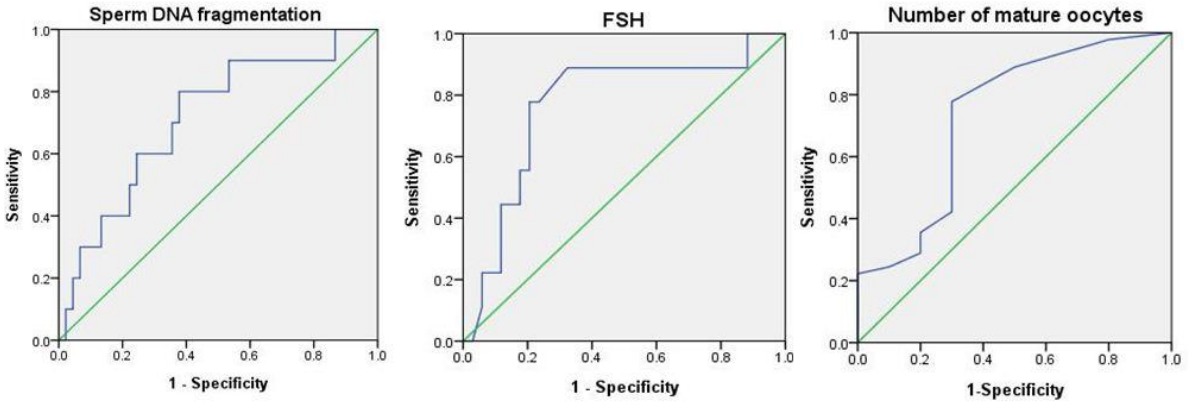

Figure 3. ROC curve analysis of sperm DNA fragmentation, serum FSH level, number of mature oocytes for prediction of miscarriage.

6. IVF outcomes according to SDF and ovarian responsiveness

Cycles with number of mature oocytes ≤3 usually indicate POR. Since high SDF level (>13%) and POR were significant factors to predict miscarriage, we divided the whole population into low SDF (≤13%) and high SDF (>13%) group and further divided them into POR group and normal ovarian responder (NOR) group (Table 9).

In the low SDF group, miscarriage rate was similar between POR and NOR group (14.2% vs. 4.3%). In the high SDF group, miscarriage rate was significantly higher in POR than NOR group (60% vs. 13.3%, p = 0.045). Pregnancy rate was not different among the four sub-groups.

Table 9. Clinical outcomes according to sperm DNA fragmentation and ovarian responsiveness

	Sperm DNA fragmentation ≤13%			Sperm DNA fragmentation >13%		
	NOR (n = 58)	POR (n = 36)	p	NOR (n = 35)	POR (n = 40)	p
Age of husband (years)	37	36	NS	37	41	0.01
Age of women (years)	34	34.5	NS	35	39	0.001
Serum level of FSH (mIU/mL)	4.9	6.6	0.012	5.9	8.1	0.007
Serum level of AMH (ng/mL)	3.6	0.8	0.001	2.6	1.1	0.001
Peak serum level of estradiol (pg/mL)	1,998	698	0.001	1,942	651	0.001
Mature oocyte	8	2	0.001	6	2	0.001
Day 3 transfer	38	29	0.001	24	38	0.002
Day 5 transfer	19	0		9	0	
Embryo transferred	2	1	0.001	2	2	NS
Grade A embryo*	1	1	0.034	1	1	NS
Grade A or B embryo*	2	1	0.002	2	1	NS
Cancelled transfer	1	7	0.009	2	2	NS
Clinical pregnancy (%)	40.3 (23/57)	24.1 (7/29)	NS	45.4 (15/33)	26.3 (10/38)	NS
Miscarriage (%)	4.3 (1/23)	14.2 (1/7)	NS	13.3 (2/15)	60 (6/10)	0.045

Expressed as median.

†p <0.05 between two groups (Mann-Whitney U test).

NOR: normal responder (no. of mature oocyte >3).

POR: poor responder (no. of mature oocyte ≤3).

*Day 3 transfer cycle only.

Discussion

In the present study, SDF level did not affect fertilization rate or pregnancy rate in IVF/ICSI cycles, while SDF level did affect miscarriage rate significantly. We found that the miscarriage rate was also affected by POR, which is generally considered as a poor prognostic factor. In this study, miscarriage rate was significantly higher (41.2% vs. 7.9%, p = 0.01) and pregnancy rate was significantly lower in the POR group (25.4% vs. 42.2%, p = 0.04), when compared with the NOR group. Nonetheless, multiple regression analysis revealed that SDF level was the only significant factor for prediction of miscarriage.

Miscarriage rate was highest in the 'POR with high SDF' group (60%), which was significantly higher than in the 'NOR with low SDF' group (4.3%) or in the 'NOR with high SDF' group (13.3%). This indicates that high SDF level contributes to miscarriage only in the POR group. Therefore, SDF testing may be of particular clinical significance for couples with POR.

When evaluating the effects of male parameters on IVF outcomes, it is important to control the female parameters, such as age, ovarian reserve and number of retrieved oocytes. Dar et al. evaluated the influence of high SDF level on fertilization, clinical pregnancy and miscarriage rate.[9]

Couples were matched by female age and serum AMH level since they could act as potential confounders. As a result, the fertilization and clinical pregnancy rates were similar between group of high SDF level (>50%) and group of low SDF level (≤15%). They showed a trend of higher miscarriage rate for the high SDF group, but it did not reach statistical significance.

Jin et al. evaluated the effect of SDF level on IVF outcomes according to ovarian reserve.[10] Reduced ovarian reserve was defined by basal FSH >10 mIU/mL, antral follicle count <6, and female age of ≥38 years. They showed that SDF level has a significant impact on clinical pregnancy and live-birth rate among women with reduced ovarian reserve, but in group of normal ovarian reserve, SDF level has no impact on clinical pregnancy and live-birth rate.

In fact, the association between SDF levels and miscarriage in IVF cycles is a conflicting issue. Two meta-analyses reported that high SDF level is associated with higher miscarriage rate,[4,5] but no association was reported in a recent meta-analysis.[6] The reasons behind this disparity are largely unknown, but different types of assay to assess DNA damage can be one of the reasons. Robinson et al. found that association between high SDF level and miscarriage was strongest when using the TUNEL assay.[4] TUNEL assay directly quantifies DNA damage by the incorporation of labeled dUTP into single- and double-stranded DNA breaks. It is generally known to have higher sensitivity and specificity for the detection of SDF over sperm chromatin dispersion (SCD) test or sperm chromatin structure assay (SCSA). A comprehensive study reported that cutoff value for defining male factor infertility was 20.1% with sensitivity of 0.764 and specificity of 0.952 when the TUNEL analysis is applied.[11]

Although one earlier meta-analysis reported no association between SDF level and fertilization rate, this topic is still conflicting.[3] Several studies reported negative effects of high SDF on fertilization rate, but studies showing no association were also reported.[9,12-17] Some studies have reported negative impact in standard IVF cycles but no impact in ICSI cycles.[16,18] In this current study, no association between SDF levels and fertilization rate was found in the whole

population and also in the five sub-groups divided according to insemination method, indications of ICSI, and semen quality, as well.

It has been reported that DNA-damaged sperms have the ability to fertilize the oocyte regardless of degree of DNA damage, but embryonic development and early pregnancy loss is closely related to the degree of damage.[19] Such finding explains the absence of association between SDF levels and fertilization rate. However, high SDF has been reported to have an adverse impact on embryogenesis. Tesarik et al. showed that high SDF is associated with 'late paternal effect' during the activation of male gene expression.[20] Sperms with highly damaged DNA would cause certain paternal genome deficiency and defective genomic activation within the embryo, and thus may have detrimental effects in late embryonic development.[21,22]

The underlying mechanisms behind the close association between higher SDF level and higher miscarriage rates in the POR group are largely unknown and require further investigation. It has been reported that oocytes possess a capability to repair damaged sperm DNA in murine models,[19] and it was suggested that the effect of SDF level on IVF/ICSI outcomes depends on oocyte quality.[23] Oocyte quality from the POR group might be poor, thus oocytes from the POR group might have poorer capability to repair damaged sperm DNA than those from the NOR group.

In the present study, high SDF level was noted in male partners from RIF couples, especially when they were less than 42 years. Our results implicate that high SDF may contribute to RIF even in the younger male population. It is still a conflicting issue whether high SDF level is associated with RIF or recurrent pregnancy loss (RPL). While some groups have reported

significantly higher SDF levels in the RPL group,[24,25] others have reported a lack of association between SDF levels and RPL or RIF.[26,27]

Limitations of this study include the retrospective nature of the study design and small sample size. We used the TUNEL assay for SDF measurement, and therefore direct comparison with SDF assessed by SCD or SCSA was not possible.

In conclusion, no association was found between SDF level and fertilization rate or pregnancy rate in IVF/ICSI cycles. However, SDF level significantly affected miscarriage rate, especially in women with POR. These findings indicate the need for SDF testing in couples with POR to provide additional information on the prognosis of pregnancy.

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국 문 초 록

목적: 정자 DNA 분절 (이하 SDF) 이 체외수정시술의 결과에 미치는 영향에 대해서 많은 논란이 있다. 본 연구는 SDF가 체외수정시술 후 수정률, 임신율, 유산율에 영향을 미치는지 알아보고자 하였다.

방법: 본 연구는 후향적 연구로서 2012년 1월부터 2014년 6월까지 분당서울대학교병원에서 시행한 169건의 체외수정시술을 대상으로 하였다. 정액은 난자채취 당일 채취되었으며, 기존 정액검사 지표와 더불어 TUNEL 기법을 이용하여 SDF를 측정하였다. 난자와 정자의 질에 따라 고식적 체외수정 또는 미세수정술이 사용되었으며 채취 3일 또는 5일 후 배아이식을 하였다. 저반응군은 채취된 성숙난자수가 3개 이하인 경우로, 유산은 12주 이전 임신이 중단된 경우로 정의하였다.

결과: SDF는 수정률과 임신율에는 영향을 미치지 않았으나 유산율과는 유의한 연관성이 있었다. 유산군 10명은 생아출산군 45명에 비하여 SDF 값이 유의하게 높았으며 (23.9% vs. 14.1%), 성숙난자수는 유의하게 적었다 (4.3 vs. 7.6). 다중회귀분석 적용시 SDF 값은 유산 예측에 대한 유의한 독립 변수로 나타났다 (OR 1.051, 95% CI 1.001 - 1.104). 유산을 예측하는 cutoff value로서 SDF 값은 >13%, 성숙난자수는 ≤3 (즉 저반응군) 이었다. SDF ≤13% 일 때 저반응군과 정상반응군 간에 유산율에는 차이가 없었으나 (14.2% vs. 4.3%), SDF >13% 일 때 유산율은 저반응군에서 정상반응군보다 유의하게 높았다 (60% vs. 13.3%, p = 0.045).

결론: 본 연구에서 높은 SDF (>13%)은 유산율의 증가와 유의한 연관성이 있었으며, 이와 같은 연관성은 저반응군에서 보다 두드러지게 나타났다. 따라서 저반응이 예상되는 환자군에서 SDF 측정이 체외수정시술을 통한 임신의 예후를 예측하는데 도움이 된다고 할 수 있겠다.

주요어: 정자, DNA 분절, 체외수정시술, 임신, 유산

학번: 2014-25040