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Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa

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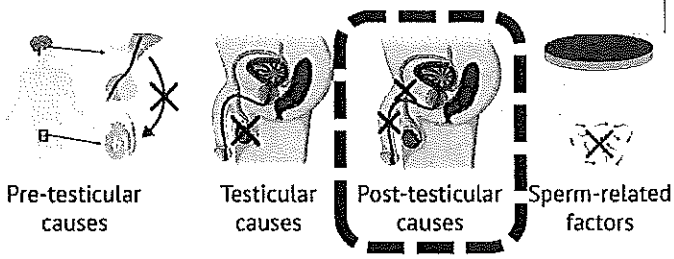
BACKGROUND: Sperm DNA damage (fragmentation) is a recently discovered cause of male infertility for which no efficient treatment has yet been found. Previous findings have suggested that clinically relevant sperm DNA damage may occur at the post-testicular level. This study was undertaken to assess the clinical usefulness of ICSI with testicular spermatozoa in this indication. METHODS: The percentage of spermatozoa with fragmented DNA, assessed by terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labelling assay, and ICSI outcomes were compared in two sequential attempts performed, respectively, with ejaculated and testicular spermatozoa in 18 men with increased sperm DNA fragmentation. RESULTS: The incidence of DNA fragmentation was markedly lower in testicular spermatozoa as compared with ejaculated spermatozoa. No differences in fertilization and cleavage rates and in embryo morphological grade were found between the ICSI attempts performed with ejaculated and with testicular spermatozoa. However, eight ongoing clinical pregnancies (four singleton and four twin) were achieved by ICSI with testicular spermatozoa (44.4% pregnancy rate; 20.7% implantation rate), whereas ICSI with ejaculated spermatozoa led to only one pregnancy which was spontaneously aborted. CONCLUSIONS: These data show that ICSI with testicular spermatozoa provides the first efficient assisted reproduction treatment option for men with high levels of sperm DNA damage.

DFI（精子 DNA 損傷インデックス）の高い男性不妊における、精巣内精子を用いた ICSI の有効性

DFI: DNA fragmentation index



背景：精子のDNA損傷は男性不妊の原因になる。この損傷は、造精後から射出までの過程（post-testicular level）で起こる。本研究では、精巣内にある射出前の精子を用いてICSIをすると、臨床成績が改善するか検討した。

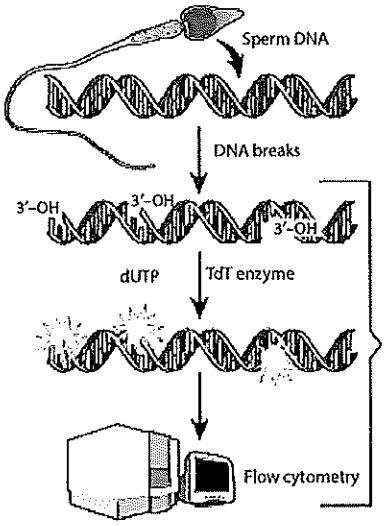
方法：DNA損傷のある精子の割合（DFI/SDF）は、Tunel assay (deoxynucleotidyl transferase-mediated dUTP nick end labeling assay) を用いて測定した。DFIの高い18症例を対象にして、射出精子と精巣内精子によるICSIの臨床成績を比較した。

結果：精巣内精子のDFIは、射出精子より顕著に低かった。受精率、分割率、初期胚のグレードに有意差はなかった。妊娠継続した8例はすべて精巣内精子のICSIによる症例であった。射出精子の妊娠例は1例のみの妊娠で流産に至った。

結論：DFIが高い場合、精巣内精子を用いたICSIは臨床成績を改善させることが示された。

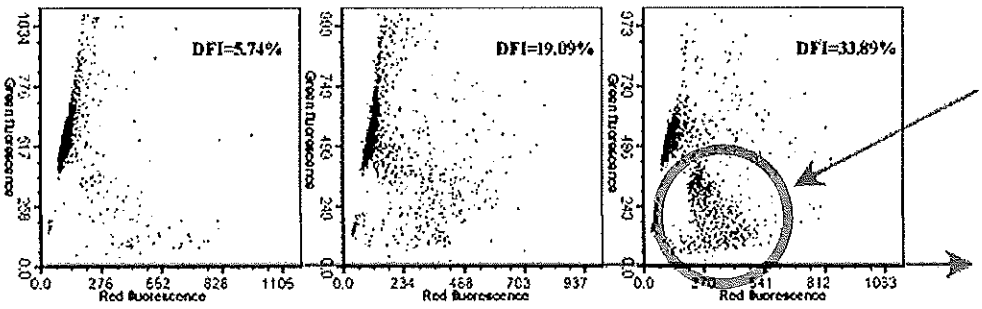
DFI測定法

Tunel assay: deoxynucleotidyl transferase-mediated dUTP nick end labeling assay



DNA損傷部位（-OH基/ヒドロキシ基）に蛍光色素を結合させたdUTPで標識

測定器（フローサイトメトリー）で蛍光を測定

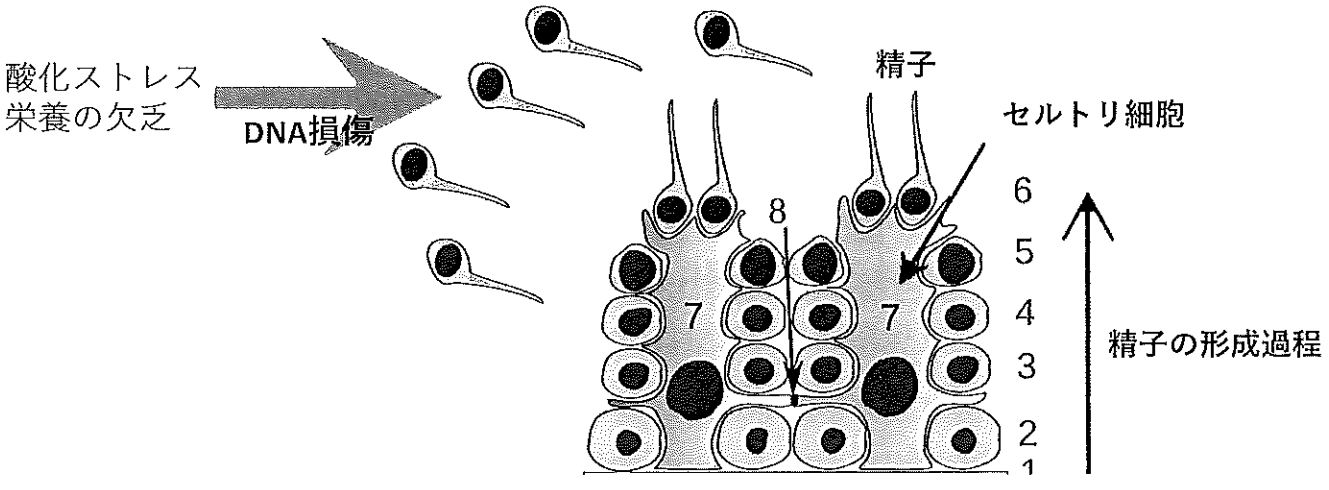


DNA損傷の高い精子

蛍光強度

背景

精子のDNA損傷は男性不妊の原因になることが知られているが、これに対する治療法はまだ確立されていない。
精子のDNA損傷はアポトーシスと似たような細胞死の機序で起こると考えられていたが、それを否定する報告もある。筆者らは、アポトーシスに似た機序は、セルトリ細胞と結合している段階の生殖細胞には起こるが、男性不妊患者で精子に生じるDNA損傷はセルトリ細胞から離れたあとに起こると報告した(Tesarik et al., 2004b)。DNA損傷の原因は、酸素ストレスやセルトリ細胞による栄養がなくなることが考えられる。
セルトリ細胞から離れた後にDNA損傷が起こるとすると、セルトリ細胞から離れて間もない精巣内精子は、射出精子よりもDNA損傷が少ないと予想される。この研究では、この仮説をもとに、精巣内精子と射出精子のDNA損傷を比較し、さらに臨床成績にも差が出るかを検討した。



方法

対象：２回以上のICSI周期で妊娠成立せず
射出精子のDFIが１５％以上
男性が非喫煙者
男性に不妊症になりうる既往歴なし
年齢　男性：２８-５５歳　女性：２４-３５歳
→１８組の不妊症カップル

・最初に射出精子によるICSIを実施し、移植不成功の場合に精巣内精子によるICSIを実施
・Long法

Table.1 精液所見と射出・精巣内精子のDFIの比較

Table I. Basic sperm parameters of the patients involved in this study and the incidence of DNA fragmentation in their ejaculated and testicular sperm samples

Patient	Basic sperm parameters			% Spermatozoa with fragmented DNA DFI	
	Concentration ($\times 10^6$/ml)	Motility (%)	Normal forms (%)	Ejaculate 射出	Testis 精巣内
1	6	11	2	20	3
2	31	52	3	15	5
3	38	71	20	24	4
4	33	40	11	21	3
5	3	19	9	27	2
6	25	65	15	31	6
7	75	42	48	25	4
8	22	3	8	23	1
9	25	15	6	22	18
10	2	14	7	25	5
11	19	29	25	26	4
12	51	41	48	21	3
13	12	63	11	37	6
14	24	32	61	19	5
15	1	42	22	17	6
16	33	21	13	24	4
17	17	56	10	20	5
18	66	44	58	27	3

射出精子のDFIはすべて≧15%だったが、精巣内精子のDFIは患者No.9以外は≦6%と著明な低下を認めた。

DFI平均値 P<0.001
・精巣内精子：4.8%
・射出精子：23.6%

精巣内精子はDNA損傷が少ない

Table.2 ラボ成績

ICSI実施卵子数　受精数　受精率　分割した胚の数（分割率）　形態な良好胚の数（良好胚率）

Table II. Fertilization and embryo development after ICSI with ejaculated and testicular spermatozoa

Sperm source	Attempts	Oocytes injected	Normal zygotes[a]	Fertilization rate[b]	Cleaved embryos[c]	Good-morphology embryos[d]
Ejaculate	18	185	131	70.8%[e]	124 (94.7%)[e]	59 (47.6%)[e]
Testis	18	187	140	74.9%[e]	133 (95.0%)[e]	68 (51.1%)[e]

[a]With two equal-sized pronuclei.
[b]Percentage of injected oocytes that developed to normal zygotes.
[c]Percentages are calculated from the number of normal zygotes.
[d]Embryos with normal pronuclear morphology on day 1, ≥6 cells on day 3, equal sized blastomeres and <10% of the intrazonal space occupied by fragments. The percentages are calculated from the number of cleaved embryos.
[e]The differences between data for the two sperm sources are not significant ($P > 0.05$).

ICSIの正常受精率、分割率、形態学的な評価（３日目初期胚）の成績に違いはなかった

Table.3 臨床成績

Table III. Implantation and pregnancy after ICSI with ejaculated and testicular spermatozoa

Sperm source	Attempts	Embryos transferred	Clinical pregnancies[a]	Pregnancy rate[b] 妊娠率	Gestational sacs[c]	Implantation rate[d] 着床率
Ejaculate 射出	18	56	1	5.6%[e]	1	1.8%[f]
Testis 精巣内	18	58	8	44.4%[e]	12	20.7%[f]

[a]With at least one gestational sac with cardiac activity.
[b]Percentage of attempts resulting in a clinical pregnancy.
[c]With cardiac activity.
[d]Percentage of embryos transferred that gave rise to a gestational sac with cardiac activity.
[e]$P < 0.05$.
[f]$P < 0.01$.

射出精子の妊娠例は１例のみで流産
精巣内精子は、臨床妊娠が８例、妊娠率44.4%、着床率は20.7%
（単胎４例、双胎４例）

Table I. Basic sperm parameters of the patients involved in this study and the incidence of DNA fragmentation in their ejaculated and testicular sperm samples

Patient	Basic sperm parameters			% Spermatozoa with fragmented DNA	
	Concentration ($\times 10^6$/ml)	Motility (%)	Normal forms (%)	Ejaculate	Testis
1	6	11	2	20	3
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3	38	71	20	24	4
4	33	40	11	21	3
5	3	19	9	27	2
6	25	65	15	31	6
7	75	42	48	25	4
8	22	3	8	23	1
9	25	15	6	22	18
10	2	14	7	25	5
11	19	29	25	26	4
12	51	41	48	21	3
13	12	63	11	37	6
14	24	32	61	19	5
15	1	42	22	17	6
16	33	21	13	24	4
17	17	56	10	20	5
18	66	44	58	27	3

・精巣内精子妊娠：下線
・射出精子妊娠：患者No. 6

精巣内精子を用いたICSIは、臨床成績を向上させる

考察

精子にDNA損傷があっても受精し胚は分割するが、移植不成功や流産につながるという過去の研究はすでに複数ある。今回の結果はその報告と合致する。

DFIの評価法にはいくつかある。sperm chromatin structure assay (SCSA)は、一本鎖DNAに結合するアクリジンオレンジ（AO）染色を用いて、フローサイトメトリーで評価する。SCAS法ではDFI＞30%でART,IUIにおいて妊娠例はなかったという報告がああり、DFI>30%をカットオフ値とする報告が多い。
Tunel法は直接DNA損傷部位（-OH基）を評価できる。Tunel法では、＞20%で妊娠例はなかったという報告がある。他の文献では、15-20%、24.3%など一定ではなく、まだ定まっていない。

精子のDNA損傷による不妊は、卵子の活性化不全や細胞分裂での有糸分裂に必要な中心小体の欠乏によるものと考えられるが、まだ明らかでない。
DFI高値の男性不妊に対して、TESE以外の方法は、抗酸化作用をもつサプリの内服やDNA損傷の少ない精子を選別する方法が期待される。

本研究では、DNA損傷による男性不妊に対する有効な新しい選択肢を初めて報告し、有効な治療法である。しかしTESEは侵襲的であり、個々の症例で慎重に適応を判断するべきである。

精巣内精子は、ラボ成績を改善させないが、臨床成績を改善させる

・精巣内精子と射出精子のDFI比較

TABLE 1

Sperm DNA assessment results.

Patient	DFI (%) Initial	DFI (%) Post-treatment	TUNEL (%) Ejaculate	TUNEL (%) Testicular	Absolute difference ejaculate versus testicular (%)
1	75.59	75.89	70.8	14.5	−56.3
2	64.15	80.85	53	32.2	−20.8
3	43.29	39.57	23.3	13.2	−10.1
4	55.99	57.89	42.7	14.8	−27.9
5	45.75	56.71	56.5	9.0	−47.5
6	53.89	54.92	43.3	8.7	−34.6
7	33.48	52.69	40.5	12.6	−27.9
8	45.5	31.77	31.5	6.6	−24.9
9	33.79	45.17	20.8	6.5	−14.3
10	58.48	51.26	36.8	7.8	−29
11	46.08	40.53	32.4	12.6	−21.8
12	35.58	38.67	25	21.7	−3.3
Mean	49.2 ± 12.8	52.2 ± 14.7	39.7 ± 14.8	13.3 ± 7.3[a]	−26.5 ± 14.8

Note: Values are mean ± standard deviation. DFI = DNA fragmentation index; TUNEL = terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling.
[a] $P < .001$.

Moskovtsev. Testicular spermatozoa DNA damage. Fertil Steril 2010.

Moskovtsev SI et al., Testicular spermatozoa have statistically significantly lower DNA damage compared with ejaculated spermatozoa in patients with unsuccessful oral antioxidant treatment. Fertil Steril. 2010 Mar 1;93(4):1142-6.

射出精子より、精巣内精子の方がDNA損傷が少ない

・卵子のDNA損傷は？
卵子にもDNA損傷は考えられるが、常に造られている精子と違って研究しにくいため、研究データはほとんどない。卵子は、卵胞に包まれているため機械的な要因によるDNA損傷は低いと思われるが、代わりに年齢的な要素でDNA損傷は損傷が蓄積される。受精後にDNA損傷の修復機構はある

Ménézo Y et al., DNA damage and repair in human oocytes and embryos: a review. Zygote. 2010 Nov;18(4):357-65.

・精子DFIの違いによる、胚盤胞到達率、良好胚盤胞到達率

TABLE 4

Effect of SDF on laboratory and clinical outcomes.

Variable	<30% SDF (n = 433)	≥30% SDF (n = 42)	*P* value
Laboratory outcomes[a]			
Fertilization rate	90.10 ± 3.50	85.67 ± 1.03	.226
Normal cleavage speed rate	72.16 ± 1.30	61.56 ± 4.40	.010
High-quality embryos at day 3 rate	36.47 ± 1.51	23.89 ± 5.51	.021
Blastocyst rate	56.25 ± 2.01	39.01 ± 1.40	.016
Blastocyst quality rate	30.54 ± 2.27	11.32 ± 7.72	<.001
Clinical outcomes[b]			
Implantation rate	46.09 ± 0.55	33.21 ± 1.96	<.001
Chemical pregnancy rate	34.99	33.11	.940
Clinical pregnancy rate	32.42	30.33	.774
Miscarriage rate	17.8	39.9	.018

[a] Adjusted for maternal age, maternal BMI, total FSH dose, number of retrieved oocytes, and paternal age.
[b] Adjusted for maternal age, maternal BMI, total FSH dose, number of retrieved oocytes, paternal age, number of transferred embryos, endometrial thickness.

Borges. Sperm DNA fragmentation and ICSI outcomes. Fertil Steril 2019.

Borges E Jr et al., Sperm DNA fragmentation is correlated with poor embryo development, lower implantation rate, and higher miscarriage rate in reproductive cycles of non-male factor infertility. Fertil Steril. 2019 Sep;112(3):483-490.

DFI低いほうが、良好胚盤胞到達率が高い

・精巣内精子と射出精子の臨床成績の比較

First International Journal of Andrology
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ORIGINAL ARTICLE

ICSI outcome in patients with high DNA fragmentation: Testicular versus ejaculated spermatozoa

M. Arafa ✉, A. AlMalki, M. AlBadr, H. Burjaq, A. Majzoub, S. AlSaid, H. Elbardisi

First published: 12 May 2017 | https://doi.org/10.1111/and.12835 | Citations: 25

Summary

本研究以外でも臨床成績について同様の報告あり

Sperm DNA fragmentation (SDF) has emerged as an important biomarker in the assessment of male fertility potential with contradictory results regarding its effect on ICSI. The aim of this study was to evaluate intracytoplasmic sperm injection (ICSI) outcomes in male patients with high SDF using testicular versus ejaculated spermatozoa. This is a prospective study on 36 men with high-SDF levels who had a previous ICSI cycle from their ejaculates. A subsequent ICSI cycle was performed using spermatozoa retrieved through testicular sperm aspiration. Results of the prior ejaculate ICSI were compared with those of the TESA-ICSI. The mean (*SD*) SDF level was 56.36% (15.3%). Overall, there was no difference in the fertilization rate and embryo grading using ejaculate and testicular spermatozoa (46.4% vs. 47.8%, 50.2% vs. 53.4% respectively). However, clinical pregnancy was significantly higher in TESA group compared to ejaculated group (38.89% [14 of 36] vs. 13.8% [five of 36]). Moreover, 17 live births were documented in TESA group, and only three live births were documented in ejaculate group ($p < .0001$). We concluded that the use of testicular spermatozoa for ICSI significantly increases clinical pregnancy rate as well as live-birth rate in patients with high SDF.

・コエンザイムQ10の効果は？

Table 2.
Seminal plasma CoQ10, oxidative stress markers, and sperm DNA fragmentation levels in fertile and infertile men before and after administration of CoQ10 and Centrum

Variable	Fertile control	Patient before CoQ10	Patient after CoQ10	Patient before Centrum	Patient after Centrum
CoQ10 level (ng/mL)	56.2±38.5	41.6±29,8[d)]	76.9±26.3[b),e)]	38.9±27.6[d)]	40.2±28.1[d),g)]
ROS (×10^4 RLU/min/20 million spermatozoa	0.07±0.03	3.52±1.29[e)]	2.68±1.31[c),e)]	2.8±0.96[e)]	2.08±1.04[c),e),f)]
TAC (mmol/L)	1.12±0.21	0.73±0.36[d)]	0.92±0.4[b),d)]	0.56±0.24[d)]	0.73±0.3[c),d),f)]
Catalase (U/mL)	12.45±2.49	8.42±2.21[e)]	9.8±2.06[b),e)]	6.72±1.75[e)]	7.8±1.4[b),e),g)]
Sperm DNA fragmentation (%)	13.2±3.8	35.2±6.4[e)]	32.1±7.9[a),e)]	28.3±5.1[e)]	25.7±4.1[b),e),g)]

CoQ10, coenzyme Q10; ROS, reactive oxygen species; TAC, total antioxidant capacity; FDR, false discovery rate.

[a)] vs. patients before CoQ10, FDR $p<0.05$;

[b)] vs. patients before CoQ10, FDR $p<0.01$;

[c)] vs. patients before CoQ10, FDR $p<0.001$;

[d)] vs. fertile control group, FDR $p<0.01$;

[e)] vs. fertile control group, FDR $p<0.001$;

[f)] vs. patients after CoQ10, FDR $p<0.01$;

[g)] vs. patients after CoQ10, FDR $p<0.001$.

有意差あるが、臨床的意義のある差なのかどうか

・PICSIは？　（ランダム化比較試験）

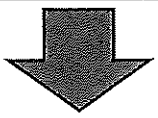
	PICSI	ICSI	Absolute difference (95% CI)	Odds ratio (95% CI)	p value
Term livebirth					
Primary analysis*	27·4% (379/1381)	25·2% (346/1371)	2·2% (-1·1 to 5·5)	1·12 (0·95 to 1·34)	0·18
Sensitivity analysis†	27·5% (379/1379)	25·3% (346/1370)	2·2% (-1·1 to 5·5)	1·13 (0·95 to 1·34)	0·17
Secondary endpoints					
Clinical pregnancy	35·2% (487/1382)	35·7% (491/1375)	-0·5% (-4·0 to 3·1)	0·98 (0·84 to 1·15)	0·80
Miscarriage	4·3% (60/1381)	7·0% (96/1371)	-2·7% (-4·4 to -0·9)	0·61 (0·43 to 0·84)	0·003
Premature birth	3·3% (46/1381)	3·3% (45/1371)	0·0% (-1·3 to 1·4)	1·02 (0·67 to 1·55)	0·94
Exploratory endpoints					
Fertilisation rate (%)‡	66% (24·0)	69% (24·0)	3·0% (-0·47 to 6·5)	1·15 (0·98 to 1·34)	0·09
Biochemical pregnancy	39·5% (546/1383)	39·5% (544/1377)	0·0% (-4·0 to 4·0)	1·00 (0·86 to 1·17)	0·99

Data are % (n/N), unless otherwise stated. PICSI=physiological intracytoplasmic sperm injection. ICSI=intracytoplasmic sperm injection. *Adjusted for maternal age, previous miscarriage, and hormonal indicators of ovarian reserve. †Adjusted for hyaluronan-sperm binding score, maternal age, previous miscarriage, and hormonal indicators of ovarian reserve. Odds ratios are shown alongside absolute differences. ‡Data are mean (SD); denominators were 1386 for the PICSI group and 1380 for the ICSI group.

Table 3: Trial outcomes

Miller D et al., Physiological, hyaluronan-selected intracytoplasmic sperm injection for infertility treatment (HABSelect): a parallel, two-group, randomised trial. Lancet. 2019 Feb 2;393(10170):416-422.

流産率を低下させるが、妊娠率を改善させない
※DFI高値の男性不妊に絞った研究ではないが



やはりDFI高い男性不妊に、精巣内精子を用いたICSIが有用か

・精巣内と射出精子による胚異数性への影響は

Table 1. Comparison of semen analyses, DNA damage, and aneuploidy rates between controls and patients.

	Controls (n = 10)	Patients (n = 8)	
Parameters	Ejaculate	Ejaculate	Testicular
Sperm concentration[a]	65.2 ± 33.5*	26.7 ± 38.8	NA
Sperm motility (%)	55.9 ± 23.3*	14.1 ± 13.6	NA
DNA damage (%)	12.1 ± 5.1*	40.6 ± 14.8	14.9 ± 5.0**
18 aneuploidy (%)	0.45 ± 0.15	1.06 ± 0.22	2.07 ± 1.23**
X/Y aneuploidy (%)	0.81 ± 0.41*	1.70 ± 0.56	5.49 ± 2.45**
13 aneuploidy (%)	0.42 ± 0.17*	1.19 ± 0.50	1.64 ± 0.83
21 aneuploidy (%)	0.66 ± 0.36*	1.60 ± 0.56	2.33 ± 0.52**
Total diploidy (%)	0.10 ± 0.06	0.23 ± 0.18	0.86 ± 0.44**
Total aneuploidy (%)	2.49 ± 0.56*	5.77 ± 1.22	12.41 ± 3.71**

Values are mean ± SD

[a] Number of sperm × 10^6/mL

*$P < 0.05$ between ejaculate of controls and patients

**$P < 0.05$ between ejaculate and testicular sample of patients

Moskovtsev SI et al., A comparison of ejaculated and testicular spermatozoa aneuploidy rates in patients with high sperm DNA damage. Syst Biol Reprod Med. 2012 Jun;58(3):142-8.

DNA損傷の低い精巣内精子は、臨床成績を上げるが、胚の染色体数的異常を減らさない
（「外見的な」染色体の本数は正常でも、「中身の」DNAレベルでは異常であることも）

PGT-Aの正常胚移植の反復不成功例は、DFIが高ければ精巣内精子を考慮？

ただし正常胚多数得られる夫婦だけを対象にするべきか