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*Original Article*

# Effect of sperm DNA fragmentation on the clinical outcomes of two assisted reproduction methods: IVF and ICSI

Jianjun Dong, Yuzhen Lv, Guangli Zhu, Fang Zhao, Juan Wang, Keke Tian, Ting Li, Yilin Liu, Yi Jiang

*Reproductive Medicine Center, Jiaozuo Maternity and Infant Health Hospital, Jiaozuo, Henan Province, China*

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**Abstract:** Objective: To investigate the effect of sperm DNA fragmentation index (DFI) on the clinical outcomes of *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Methods: Infertile couples were prospectively recruited in the Reproductive Center in Jiaozuo Maternal and Child Care Service Centre in Henan, China, from July 2015 to December 2016. All the recruited 228 infertile couples were divided into IVF group (135 cycles) and ICSI group (100 cycles) in accordance with what kinds of assisted reproductive technology (ART) they had chosen. Firstly, DFI of males in the two groups were detected by sperm chromatin structure analysis (SCSA). Then, the semen samples were collected on the day of oocyte retrieval of female spouses and performed the routine semen analysis and DFI testing. Finally, according to DIF level, the two group patients were divided into three groups: Group A (DFI<8%), Group B (8%≤DFI<15%), Group C (15%≤DFI<25%). The rates of fertilization, embryo cleavage, good quality embryos, embryos implantation and clinical pregnancy in VFI and ICSI cycles were analyzed and compared among three groups. Results: During the normal treatment cycle, when DFI≤15%, there was no statistical difference between IVF and ICSI in the rates of fertilization, embryo cleavage, good quality embryos, embryos implantation and clinical pregnancy (P>0.05). When 15%≤DFI<25%, the rates of fertilization and clinical pregnancy rates in ICSI were significantly higher than those in IVF (P<0.05), but there was also no significant difference between IVF and ICSI in good quality embryos rate (P>0.05). Conclusion: The rise of DFI level can not only reduce the sperm motility but also affect the clinical outcomes of IVF and ICSI. While the outcomes of IVF and ICSI are statistically similar when DFI level is less than 15%, the curative effect of ICSI is better when DFI value exceeds 15%.

**Keywords:** Sperm DNA fragmentation, in vitro fertilization, intracytoplasmic sperm injection

## Introduction

Sperm DNA fragmentation (SDF) is also called sperm DNA damage. It has been confirmed that level of SDF is significantly higher in semen of infertile males. Therefore, the integrity of sperm DNA fragmentation is not only the effective evaluation index to evaluate the quality of sperm, but also the independent clinical and biological indicator to reflect the male fertility [1-3]. Researches have shown that a high level of SDF may affect the natural fertility, the fertility of internal fertilization (such as intrauterine insemination), and the curative effect of assisted reproductive technology (ART) [4-6]. Consequently, these results can direct the application of ART. Nowadays, this problem still has arguments, which might be caused by the different level of SDF, but it is an important issue when IVF and ICSI are widely applied in the male infertility of sperm abnormality. This study explored the correlation between the different levels of SDF and the clinical outcomes of *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI).

## Materials and methods

### *Clinical materials*

*Patients:* Two hundred and twenty-eight couples, who were treated with IVF and ICSI for 235 ART treatment cycles in the Reproductive Center in Jiaozuo Maternal and Child Care Service Centre in Henan, China, were enrolled in this study. They were included according to the criteria of relevant literature [7-9]. Inclusion criterion: Couples with normal karyotype after the detection of chromosome technique; couples with normal ART (IVF/ICSI) cycles; female

**Table 1.** The basic information of IVF and ICSI

| Group | Cycle number (n) | Paternal age (year) | Infertility age (year) | Sperm density (*$10^6$/ml) | Normal sperm shape (%) | Sperm motility (%) |
|---|---|---|---|---|---|---|
| IVF | 135 | 32.5±5.2 | 3.3±1.8 | 68.8±32.5 | 8.7±3.8 | 56.9±11.2 |
| ICSI | 100 | 33.4±4.4 | 3.6±1.9 | 65.5±30.1 | 8.2±4.1 | 55.2±10.9 |

spouses with normal ovarian function whose infertility was purely due to tubal factor, and aged less than 38 years old; the sperm count of fresh semen samples in male spouses was more than 1*$10^5$/ml. Exclusion criterion: Male spouses with severe oligoasthenospermia; female spouses who had suffered polycystic ovary syndrome, endometriosis, hyperprolactinemia, hydrosalpinx. All the patients signed the informed consent before the operation.

*Instrument and reagent:* Computer-assisted sperm analysis (CASA-WLIT-9000) from Beijing Weili Company was adopted. BD FACSCalibur was applied for SDF detection. DFI reagent was purchased from Zhejiang Cellpro Biotech Co., Ltd.

*Methods*

*Collection of semen samples and routine semen analysis:* According to the standard of World Health Organization (WHO), male spouses were required to be abstinent for 3-5 days and obtained semen by the method of masturbation and put it in the sterile cup on the day of oocyte retrieval of female spouses. The semen samples were liquefied at room temperature of 25°C. The 0.5 ml semen of each sample was add into cryovial and then preserved in the liquid nitrogen. Meanwhile, the routine semen analysis was performed to these samples according to the standard of WHO. The measure indicators included sperm quantity (ml), sperm density (*$10^5$/ml), sperm motility and normal sperm shape.

*SDF detection:* Sperm chromatin structure analysis (SCSA) and flow cytometry were used to detect SDF. The detection principle and operational approach were in accordance with the methods proposed by Jurewicz et al. [10-12]. After acid treatment of semen, the sperm DNA which had structural damage would be unwinded from double stranded structure to single-stranded one, and then could glow red fluorescence after combining with acridine orange and presenting a form of polymer. While the normal sperm DNA still kept an intact double-stranded structure, it would glow green fluorescence after combining with acridine orange and presenting a form of monomer. Afterwards, BD flow cytometry was used to detect the signal of fluorescence. Having sperm denatured and stained, if the proportion of red fluorescence increased, it could be concluded that the damage extent of sperm integrity also increased. The DNA fragmentation index (DFI) value was calculated by CASA-WLJW-9000. The formulation was as follows: DFI value of single sperm = red/(red + green) *100%. It represented the ratio of denatured sperm DNA in the total DNA. DFI value of sperm group was expressed as the proportion of ultra-group sperm, representing the ratio of damaged sperm in total sperm. It was important to note that at least 5000 sperm should be tested in each sample and detected continuously at least 2 times.

*IVF and ICSI treatment:* The routine superovulation strategy was adopted in this study. The follicular development was detected with B-scan ultrasonography and estradiol level monitoring. As the follicle matured, human chorionic gonadotropin (hCG) was injected intramuscularly, and oocytes were taken out after 36 hours. IVF and ICSI treatments were performed at the same day of oocyte retrieval and the cleavage conditions were observed after 48 and 72 hours. The modified Dab method was used to evaluate the embryos, which were divided into stage I, II, III, IV. Stage I and II represented embryos were in high quality. Two to three high quality embryos were selected for transplantation. Four weeks after transplantation, patients whose urine and blood hCG were positive at day 14 should take B-scan ultrasonography. It could be recognized as clinical pregnancy when intrauterine gestational sac was observed.

*Statistical analysis*

SPSS17.0 statistical software was adopted for statistical analysis. The measurement data was presented as mean ± standard deviation ($\bar{x}$±s). The enumeration data was expressed as rate. The independent-samples t test was used

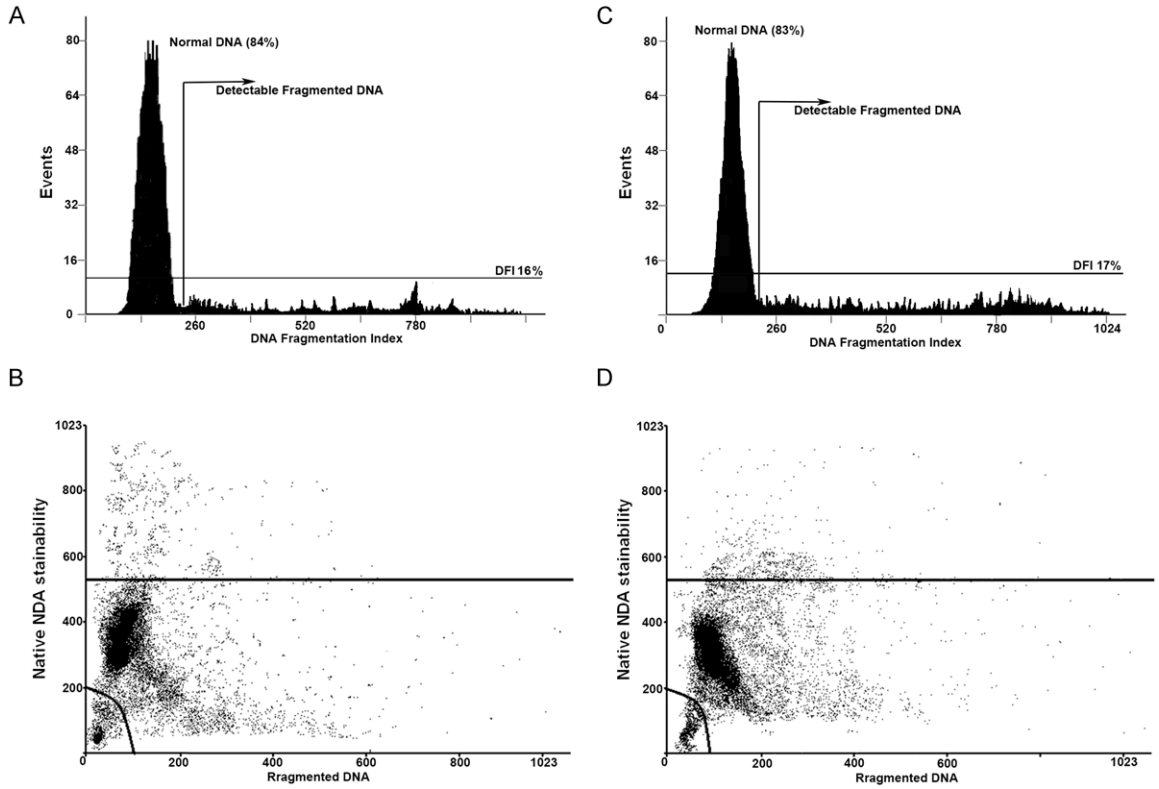


**Figure 1.** DFI features of IVF cycle and ICSI cycle. (A, C) Sperm chromatin structure analysis frequency distribution histogram of the DNA fragmentation index (DFI) of IVF cycle (A) and ICSI cycle (C); (B, D) SCSA scattergram of fragmented DNA (red fluorescence) *vs* native DNA stainability (green fluorescence) of the same semen sample of IVF cycle (B) and ICSI cycle (D), and the long line on the y-axis (channels 550°) indicates the threshold for high DNA stainability. SCSA, Sperm chromatin structure analysis.

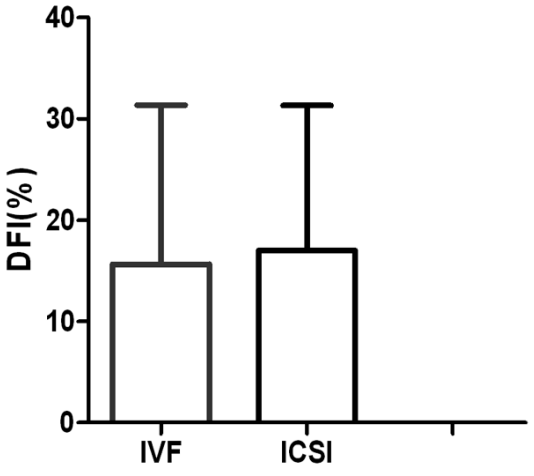


**Figure 2.** DFI features of IVF and ICSI treatment cycles.

for comparison between groups. The single factor analysis of variance combined with post hoc Bonferroni was used for the comparison among 3 groups of data. The $X^2$ test was used to compare the rates of two groups. P<0.05 was considered statistically significant.

## Results

### *Basic situation of ART cycles*

There were 135 IVF cycles and 100 ICSI cycles in 235 selected cycles. The sperm density, sperm motility and normal sperm shape of IVF cycles were all higher than ICSI cycles. The duration of infertility of ICSI cycles was longer than IVF cycles. However, these differences were not statistically significant (P>0.05), as shown in **Table 1**.

### *DFI features of ART cycles*

The average value of DFI was (15.6±9.5)% in the IVF cycles, (17.2±10.5)% in the ICSI cycle. Therefore, the value of DFI was slightly higher in the ICSI cycles. The difference was not statistically significant (P>0.05, **Figures 1**, **2**).

**Table 2.** Comparison of general conditions and semen routine parameters of patients with different DFI values in IVF group

| Group | n | Male age (years) | Infertility years (year) | Sperm density (*$10^6$/ml) | Sperm motility (%) | Normal shape (%) |
|---|---|---|---|---|---|---|
| A | 52 | 34.6±3.8 | 3.5±1.8 | 55.3±33.9 | 68.1±15.6 | 8.3±3.7 |
| B | 40 | 29.3±4.1 | 3.4±1.9 | 58.5±35.6 | 68.5±20.7 | 8.6±4.9 |
| C | 43 | 31.9±3.9 | 3.1±2.0 | 57.6±30.8 | 69.4±32.2 | 8.4±3.5 |

Note: Comparison among three groups, P>0.05.

**Table 3.** Comparison of general conditions and semen routine parameters of patients with different DFI values in ICSI group

| Group | n | Male age (year) | Infertility years (year) | Sperm density (*$10^6$/ml) | Sperm motility (%) | Normal shape (%) |
|---|---|---|---|---|---|---|
| A | 32 | 33.1±3.7 | 3.8±1.5 | 56.2±30.9 | 67.5±14.6 | 8.2±4.7 |
| B | 33 | 28.8±4.3 | 3.6±1.9 | 57.5±29.6 | 68.4±16.7 | 8.3±3.8 |
| C | 35 | 33.9±4.2 | 3.2±2.2 | 58.7±28.1 | 69.1±27.2 | 8.5±3.7 |

Note: Comparison among three groups, P>0.05.

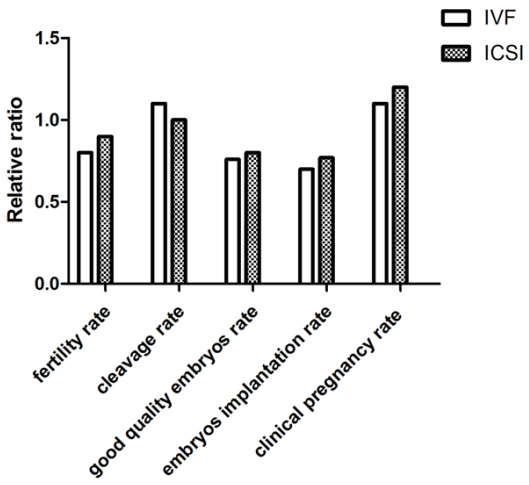


**Figure 3.** The treatment outcomes of ICSI and IVF (DFI<8%).

*Basic information of ART cycles with different DFI*

Normal reference value: DFI<15%. The two ART cycles were divided into group A (DFI<8%), group B (8%<DFI<15%) and group C (15%< DFI<25%), as shown in **Tables 2** and **3**.

*Comparison of the embryo outcomes with IVF and ICSI at different SDF levels*

The embryo outcomes treated with IVF and ICSI at different SDF levels were compared among group A, B and C. The results showed that there was no statistical difference in the rates of fertilization, good quality embryos, embryos implantation and clinical pregnancy between the two fertilization methods when the SDF level was low (DFI<15%, P>0.05), while the embryo outcomes of IVF and ICSI cycles had no significant differences (DFI<15%, P>0.05), as shown in **Figures 3** and **4**. When SDF level was comparatively higher (15%≤DFI<25%), the rates of fertilization, cleavage, embryos implantation and clinical pregnancy in IVF treatment were significantly decreased compared with ICSI treatment (P<0.05) (**Figure 5**). However, regardless of different SDF levels, there was no statistical significance in the good quality embryos rate between IVF treatment and ICSI treatment (P>0.05), as shown in **Figures 4** and **5**.

## Discussion

SDF is a kind of defect of the genetic integrality of sperm and is a valid indicator for the evaluation of male fertility [13, 14]. Numerous studies have confirmed that sperm DNA fragmentation can predict the treatment outcomes of ART and guide therapies [15-18]. Some studies have suggested that the increase level of DFI can reduce the motility and density of sperms and change the normal forms of sperms. The possible mechanism is that the high level of SDF leads to the mutation of the gene of the sperm $Na^+$-$K^+$-ATPase, which causes the denaturation of ATPase and finally results in the reduction of sperm motility, sperm density and normal morphological rate [19, 20]. Therefore, it can be concluded that SDF levels are negatively correlated with sperm motility. However, this study did not agree with this point. Our study found that the DFI value of ICSI cycle was higher than that of IVF cycle, and the corresponding sperm concentration, sperm motility and normal sperm forms were slightly lower than the IVF cycle, but the difference was not statistically significant, suggesting that sperm DNA damage might not significantly change the general parameters of sperms, which was different from the results of previous researches. It was speculated that the occurrence of different results

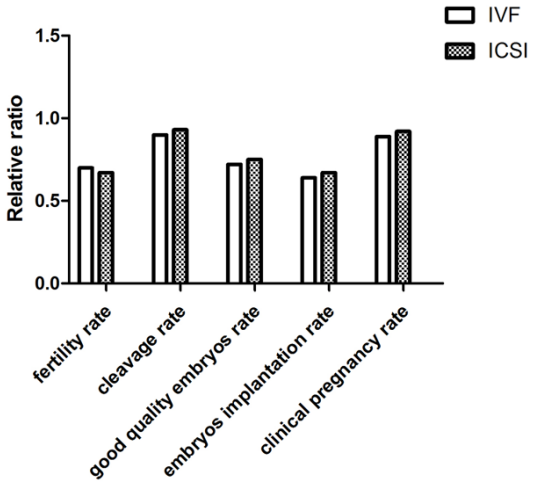


**Figure 4.** The treatment outcomes of ICSI and IVF (8%<DFI<15%).

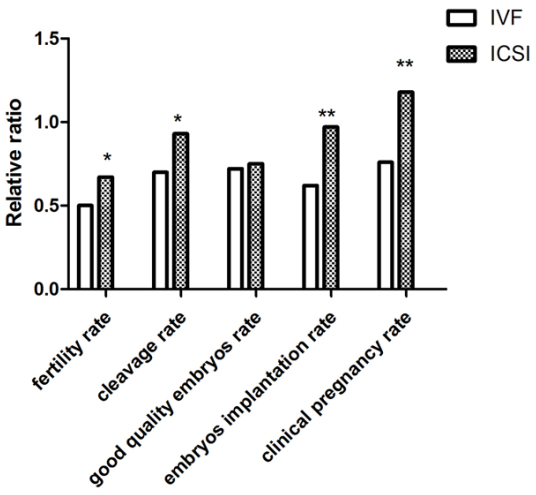


**Figure 5.** The treatment outcomes of ICSI and IVF (15%<DFI<25%). Compared with IVF group, *P<0.05, **P<0.01.

might be related to the different patients recruited in the study. First, the sample size of this research was small. Second, as for couples who adopted ART mainly because of the oviduct factors, the correlation between DFI and sperm parameters might not exist. Moreover, the grouping of this experiment was based on the level of DFI, therefore, even though the abnormal DFI might lead to the change of general parameters of spermatozoa, but the marginal value of DFI was unclear. Therefore, the results of this study might be biased to some extent. And further studies with larger sample size should be done to identify the correlation between DFI and sperm parameters and determine the criteria of the normal range of DFI.

The DFI value of the sperm samples was mainly distributed between 8% and 25% in the 235 ART cycles of this study, while it was mainly distributed between 0% and 10% in ART cycles of studies conducted by Simon et al., which might be mainly caused by different SDF detection methods [21-23]. The SCSA was used in this study to detect the integrity of sperm nucleus, while sperm chromatin diffusion (SCD) assay and terminal deoxynucleotidyl transferase-mediated dUTP biotin nick end labeling (TUNEL) were used in the latter. Different SDF detection methods resulted in differences in the main distribution interval of sperm DFI in the ART cycles. SCD assay has been widely used in various studies for defining the threshold value of SDF. However, the threshold values are varied due to different people and the artificial reproduction technology, including 20%, 27%, 22% and 30%. As for the detection of sperm DNA damage, TUNEL is considered as the most potential and standardized testing method. Many studies have showed that its threshold is from 4% to 25%.

In the field of ART treatment, agnate genetic material carried by human sperm DNA is completely transmitted to the next generation. If the sperm DNA is defective due to the impact of some congenital and acquired factors, it will inevitably affect the treatment outcome of ART [5, 21, 22]. But, at present, there is no definite conclusion about the influence of sperm DNA damage on ART treatment. Some studies have suggested that SDF only affect the outcome of ICSI treatment [19, 24, 25], because the proportion of sperms with DNA damage can be increased when treated with ICSI. However, during the IVF treatment, the zona pellucida of oocyte can automatically identify and screen out those sperms with severe DNA deficiency, therefore, the fertilized sperms are mostly the ones without damage or with little damaged DNA. Therefore, it will not affect the combination and development of sperms and oocytes after fertilization, and IVF outcome won't be affected by SDF. However, in this study, it was found that there were no significant differences in the rates of fertilization, embryo cleavage, embryo implantation, clinical pregnancy and good quality embryos between ICSI and IVF cycles when SDF level was low. But when DFI value exceeded 15%, the rates of fertilization, implantation and clinical pregnancy in ICSI cycles were significantly higher than those in

IVF cycles. Therefore, this study suggests that the impact of SDF on the treatment of ART may be among a certain threshold. When the SDF level is below the threshold, there is no significant difference between ICSI and IVF treatment outcomes, when the SDF level exceeds it, SDF has a prominent effect on the embryonic outcomes of ICSI and IVF, and ICSI outcome is better than IVF. Zini et al. also confirmed this view that when the sperm DFI value exceeded 27%, biochemical pregnancy rate of ICSI treatment was significantly higher than that of IVF treatment, while when the sperm DFI exceeded 30%, clinical pregnancy rate and delivery rate in ICSI treatment were obviously higher than those in IVF treatment [24]. In this regard, Zhang et al. [26] argued that ICSI treatment could make sperms skip the natural fertilization process and directly got into the oocyte, and then fertilized with oocytes normally, even though these sperms had DNA defect. After fertilization, oocytes could repair damaged sperm DNA. Therefore, the fertilization rate and other outcomes of ICSI treatment would not be affected. But the embryonic outcomes of IVF treatment would be affected by the failure of natural fertilization because of severe sperm DNA damage, or affected by the abnormal fertilization when the degree of sperm DNA damage after fertilization far exceeded the repair capacity of oocytes [27, 28]. Hence, when SDF exceeded an abnormal threshold, the suffered damage of sperm DNA in ICSI treatment was less than that in IVF treatment and its treatment outcome was better than that of IVF. As a result, when sperm DNA damage was serious and DFI was relatively higher, ICSI could achieve a better clinical outcome than IVF.

This study also revealed that, regardless of different levels of sperm DNA damage, there was no significant difference in good quality embryo rate between the treatment of ICSI and IVF, indicating that sperm DNA damage did not affect the good quality embryo rate of ART. However, most studies have showed that sperm DNA damage are in negative correlation with good quality embryo rate of ART [29, 30], which is different the result of this study. The possible reason is that there are only 10 cases of samples marked as abnormal in the enrolled samples, (the sample can be marked as abnormal when DFI>25%), which is too small; it is also possible that the rating outcome based on current embryonic quality classification standard is not objective and accurate enough, indicating that more advanced and accurate classification standard should be explored to better predict the clinical outcomes of ART.

In conclusion, the effect of SDF on the clinical outcome of ICSI and IVF is related to its threshold. Only when sperm DNA damage is serious and SDF reaches a certain level will the SDF have an impact on the treatment of ART; and the treatment outcome of ICSI in this study was better than that of IVF. Therefore, ICSI treatment should be chosen when the level of SDF is relatively high in ART treatment.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Yuzhen Lv, Reproductive Medicine Center, Jiaozuo Maternity and Infant Health Hospital, No.158 Minzhu Middle Road, Jiaozuo 454150, Henan Province, China. Tel: +86-138-39158938; E-mail: lvyuzhen0519@163.com

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