Sperm DNA fragmentation index and high DNA stainability do not influence pregnancy success after intracytoplasmic sperm injection

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Objective: To evaluate the ability of sperm DNA fragmentation index (DFI) and high DNA stainability (HDS) to influence the chance of achieving pregnancy in couples undergoing intracytoplasmic sperm injection (ICSI) cycles.

Design: A retrospective study evaluating couples who underwent an ICSI cycle between 2009 and 2018.

Setting: Reproductive center.

Patient(s): Consecutive couples who underwent an ICSI cycle and had a semen analysis with subsequent DFI and HDS testing, evaluated using Sperm Chromatin Structure Assay.

Intervention(s): Measurement of DFI and HDS prior to ICSI cycle.

Main Outcome Measure(s): To determine whether DFI or HDS of sperm was predictive of the number of ICSI cycles until the first clinical intrauterine pregnancy.

Result(s): A total of 550 couples who underwent 1,050 ICSI cycles were analyzed. Of those, a total of 330 couples achieved pregnancy. As expected, in couples who achieved pregnancy, females were younger and underwent fewer cycles. Importantly, the DFI and HDS were similar between couples who achieved pregnancy (DFI% 12.9; HDS% 9.3) and couples who did not (DFI% 12.2; HDS% 9.1). A multivariable-adjusted analysis evaluating female age at the first cycle was associated negatively with pregnancy.

Conclusion(s): Neither DFI nor HDS at baseline influenced the chances of a couple to achieve pregnancy after ICSI. Increased female age and couples who underwent more ICSI cycles were associated with lower chances of achieving pregnancy. (Fertil Steril Rep® 2020;1: 233–8. © 2020 by American Society for Reproductive Medicine.)

Key Words: DNA fragmentation, high DNA stainability, ICSI, sperm

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sperm DNA fragmentation impairing testicular spermatogenesis and steroidogenesis, epididymal sperm maturation, and fertilization (8, 9). Additionally, defective spermatogenesis affects sperm DNA integrity (10). Therefore, it has been suggested that sperm DNA damage might play a role in predicting the success of IVF/ICSI (11–16). This has led to the creation of tools that assess sperm DNA quality such as the sperm chromatin structure assay (SCSA). SCSA yields two main sperm variables: DNA fragmentation index (DFI), which measures abnormal chromatin structure, and high DNA stainability (HDS), which measures the amount of sperm in a semen sample having increased the amount of retained histones due to the lack of full protamination (11, 13). These parameters have been used to characterize sperm chromatin (protein) defects (13). Several studies have investigated the role of the DFI and HDS in IVF/ICSI. Some studies suggest that increases in the DFI affect pregnancy rate (17). Elevated HDS has been shown to predict pregnancy failure (18) and is associated with miscarriage (15). However, other studies suggest that there is no association between DFI and pregnancy rates, early abortion, oocyte fertilization, or quality of embryos (19). Furthermore, it has been suggested that current methods for sperm DNA fragmentation analysis have a low capacity to predict IVF/ICSI outcomes (12). Given that ICSI success has remained <30% per cycle (20) and the paucity of evidence regarding the role of HDS and DFI on ICSI, we analyzed the effect of DFI and HDS at baseline and their capacity to predict the number of ICSI cycle attempts that a couple must undergo until pregnancy is achieved. We hypothesized that the DFI and HDS at baseline will affect the number of ICSI cycles until first pregnancy is achieved and might provide an additional parameter in setting expectations for couples before starting ICSI.

**Materials and Methods**

**Study Design and Population**

We conducted a retrospective study evaluating couples who underwent an ICSI cycle between January 2009 and December 2018 at a high-volume reproductive center in Miami, Florida. All couples who underwent an ICSI cycle during this time had semen analysis and SCSA testing done for the male counterpart. Using the Society for Assisted Reproductive Technology database, all couples who underwent an ICSI cycle during this time were identified and the number of cycles they underwent was recorded as well as the outcomes for each cycle. Couples were excluded if they previously underwent ICSI, they were missing data, they had the cycle canceled for any reason, and those using donor eggs or donor semen. Men with azoospermia or severe oligospermia due to Klinefelter syndrome and Y-chromosome microdeletion and those who required surgical sperm retrieval also were excluded. In couples who achieved pregnancy, the number of ICSI cycles were considered until the first clinical intrauterine pregnancy; subsequent pregnancies were not considered in this analysis. This study was submitted to and approved by the institutional review board of the University of Miami, Miller School of Medicine, Miami, Florida.

**Primary and Secondary Outcomes**

The primary outcome of our study was to determine whether DFI of sperm was predictive of the number of ICSI cycles until success, defined as the first clinical intrauterine pregnancy. The secondary outcome was whether HDS could predict the number of ICSI cycles until success.

**SCSA Analysis**

SCSA diagnostics required a minimum of 0.20 mL with a concentration of ≥0.5 million/mL. The frozen semen was then thawed in a 37°C water bath diluted with TNE (Tris and EDTA) buffer solution. The semen sample was treated subsequently with 400 μL of acid (pH1.20) for 30 seconds to denature DNA at the sites of strand breaks. The acridine orange staining solution was added to stain the sites of single-strand DNA breaks red and the double-strand DNA breaks green. Using flow cytometry twice, the sperm cells were measured with ≥5,000 sperm each time. The DFI was calculated using measurements of the red fluorescence, divided by the total amount of red and green fluorescence together. The HDS was calculated based on the sperm population in the semen sample that has an abnormally high level of DNA staining resulting from a lack of full protamination, indicating an increased amount of retained histones, which are indicative of the amount of sperm chromatin defects. As part of a standardized clinical practice, the semen sample that was collected for SCSA analysis was obtained in an office visit prior the appointment in which the sample for ICSI was collected.

**Oocyte Retrieval**

Couples undergoing a fresh embryo transfer received a gonadotropin–releasing hormone antagonist short protocol; for couples undergoing a frozen-thawed embryo transfer, a gonadotropin–releasing hormone agonist suppression long protocol was used. Follicle-stimulating hormone (menopur) was used for ovarian stimulation with the dosage based on the patient’s baseline follicle-stimulating hormone, female age, body mass index, and ovarian volume. A transvaginal ultrasound was used to monitor ovarian response to determine the size and count of follicles. Oocyte retrieval was done using a transvaginal ultrasound probe and a 53/4 Pasteur pipette.

**Statistical Analysis**

Statistical analysis was performed with SPSS version 24 software for Windows (IBM). Means ± standard deviations or medians and interquartile ranges were reported according to the data distribution, and comparison of continuous variables was performed using the Mann–Whitney U or Student t test as required. Categorical variables were analyzed with a chi-square test. Additionally, a univariable and multivariable risk analysis was performed to determine the association (odds ratio [OR]) between pregnancy and the clinical characteristics of the couple, initial DFI and HDS category, and the number of cycles attempted. The median number of cycles until achieving first clinical pregnancy and their 95%
confidence interval (95% CI) were obtained through a Kaplan-Meier analysis, and a log-rank test was used to assess differences in the number of cycles among DFI and HDS-groups. \( P < .05 \) was considered statistically significant.

**RESULTS**

A total of 550 couples who underwent 1,050 IVF/ICSI cycles were analyzed; of those, a total of 330 couples achieved pregnancy. In those couples who achieved pregnancy, females were younger at the start of the cycles (no pregnancy 35.3 ± 3.4 years vs. pregnancy 33.7 ± 3.6 years; \( P < .001 \)) and underwent fewer cycle attempts (no pregnancy 2 [1–3] years vs. pregnancy 2 [1–2]; \( P = .001 \)). The DFI and HDS were similar between those who achieved pregnancy and those who did not (\( P > .05 \)). The rest of the clinical and demographic variables are presented in Table 1. This cohort had a mean fertilization rate of 78%, a blastocyst formation rate of 42%, and an embryo use rate of 35%.

After performing a univariable and a multivariable-adjusted risk analysis, it was shown that female age at the first cycle was associated negatively with pregnancy (OR 0.827; 95% CI 0.778–0.879; \( P < .001 \)), and couples who underwent more ICSI attempts had lower probability of getting pregnant (OR 0.597; 95% CI 0.493–0.723; \( P < .001 \)); neither the DFI or HDS were associated with an increase in chance of achieving pregnancy (\( P > .05 \)) (Table 2). We then performed a Kaplan-Meier analysis, which showed that the median number of ICSI cycle attempts until half of the couples achieved pregnancy was two cycles (95% CI 1.84–2.16), with no significant difference in accordance with the categorized DFI (\( P = .632 \)) or HDS (\( P = .692 \)) (Table 3 and Fig. 1). Furthermore, it was observed that in the first cycle that 156 couples achieved pregnancy (28.4%; 156/550). In the second cycle, an additional 122 couples achieved pregnancy (cumulative success 50.5%; 278/550) and in the third cycle an additional 42 achieved pregnancy (cumulative success 58.2%; 320/550). The fourth cycle yielded an additional eight pregnancies (cumulative success 59.6%; 328/550), the fifth cycle yielded one additional pregnancy (cumulative success 59.8%; 329/550), and in the seventh cycle one additional couple achieved pregnancy (cumulative success 60%; 330/550).

**DISCUSSION**

In our study, we evaluated 1,050 ICSI cycles in 550 couples. In couples who achieved pregnancy, the female was younger and underwent fewer cycle attempts. There were no

### TABLE 1

| Characteristic                                      | Overall | No pregnancy | Pregnancy | \( P \) value |
|-----------------------------------------------------|---------|--------------|-----------|--------------|
| No. of couples                                      | 550     | 220          | 330       |              |
| No. of cycles                                       | 1,050   | 480          | 570       |              |
| Male age (y) at first cycle                         | 37.7 ± 6.5 | 37.9 ± 6.3  | 37.5 ± 6.7 | .491        |
| Male prior fertility (%)                            | No      | 344 (62.5)   | 141 (64.1) | 203 (61.5)  |
|                                                     | Yes     | 206 (37.5)   | 79 (35.9)  | 127 (38.5)  | .541        |
| Female age (y) at the first cycle                   | No. 34.3 ± 3.6 | 35.3 ± 3.4  | 33.7 ± 3.6 | < .001      |
| Female BMI (kg/m²) at the first cycle               | No. 22.6 (20.4–25.8) | 22.5 (20.2–25.6) | 22.7 (20.5–26.5) | .499        |
| Male smoking history (%)                            | No      | 467 (84.9)   | 185 (84.1) | 282 (85.5)  | .662        |
|                                                     | Yes     | 83 (15.1)    | 35 (15.9)  | 48 (14.5)   | .473        |
| Varicocele history (%)                              | No      | 523 (95.1)   | 213 (96.8) | 310 (93.9)  |
|                                                     | Yes     | 27 (4.9)     | 7 (3.2)    | 20 (6.1)    | .126        |
| TMSC (10⁶ motile sperm)                             | No. 43.2 (9.9–101.5) | 39.4 (9.9–95.3) | 45.9 (9.9–107.6) | .483        |
| Sperm morphology (% normal forms)                   | No. 3 (1–6) | 3 (1–7)      | 1 (3–6)    | .735        |
| DFI (%)(continuous)                                 | 12.7 (7.8–20) | 12.2 (7.1–20.2) | 12.9 (8–20) | .735        |
| DFI (%)(categorized)                                | \( \leq 15 \) | 343 (62.4)   | 139 (63.2) | 204 (61.8)  |
|                                                     | 15.1–20 | 72 (13.1)    | 26 (11.8)  | 46 (13.9)   |
|                                                     | 20.1–25 | 35 (6.4)     | 12 (5.5)   | 23 (7)      |
|                                                     | 25.1–30 | 42 (7.6)     | 16 (7.3)   | 26 (7.9)    |
|                                                     | \( \geq 30.1 \) | 58 (10.5)   | 27 (12.3)  | 31 (9.4)    | .723        |
| HDS (%) (continuous)                                | 6.4 (9.2–14.4) | 9.1 (6.7–14) | 9.3 (6.1–14.6) | .914        |
| HDS (%) (categorized)                               | \( \leq 7.5 \) | 199 (36.2)   | 81 (36.8)  | 118 (35.8)  |
|                                                     | 7.6–10  | 113 (20.5)   | 42 (19.1)  | 71 (21.5)   |
|                                                     | 10.1–1  | 114 (20.7)   | 48 (21.8)  | 66 (20)     |
|                                                     | \( \geq 15.1 \) | 124 (22.5)  | 49 (22.3)  | 75 (22.7)   | .887        |
| ICSI attempts or attempts until pregnancy, median (range) | 2 (1 – 2) (1 – 10) | 2 (1 – 3) (1 – 10) | 2 (1 – 2) (1 – 7) | .001        |

*Note: Data presented as mean ± standard deviation and median (interquartile range 25–75), unless stated otherwise. BMI = body mass index; DFI = DNA fragmentation index; HDS = high DNA stainability; ICSI = intracytoplasmic sperm injection; TMSC = total motile sperm count. Blachman-Braun. Pregnancy success after ICSI. Fertil Steril Rep 2020.*
significant associations between initial DFI and HDS category and those who achieved pregnancy. Overall, the cumulative success for couples was 60% (330 couples who achieved pregnancy/550 analyzed couples); on the first cycle success was 28.4% and cumulative success for the second cycle was 50.5%. The Kaplan-Meier analysis further corroborates this observation, showing the median number of cycle attempts until pregnancy was ~2 cycles. This finding was similar for all DFI- and HDS-stratified groups, suggesting that an initial evaluation of DFI and HDS has limited potential to predict the number of attempts that a couple might undergo before pregnancy is achieved.

Our findings are comparable with previous studies. We found that females who achieved pregnancy were younger and these results are similar to the results of Liang et al. (21), who reported that female age is a predictor for successful pregnancy after IVF. In 2018 Pacey et al. (22) concluded, after reviewing nine meta-analyses that evaluated DFI and pregnancy, that there remains little consensus about the use of DFI and whether it adds clinical value. Additionally, many studies have not found any difference between DFI and HDS to achieve pregnancy (12, 15, 16). However, Borges et al. (23), in a study of 445 cycles analyzed for DFI, found that DFI corresponded with negative outcomes for pregnancy, with a cut-off value for DFI at >30%. In our study, couples with a DFI >30% had similar outcomes to other couples and underwent similar number of ICSI attempts as couples less percentage of initial DFI.

| TABLE 2 |
| --- |

| Characteristic | Univariable | Multivariable |
| --- | --- | --- |
| Male age (y) at first cycle | 0.991 | 1.012 | 0.491 | 0.980–1.045 | 0.463 |
| Male prior fertility (%) | No | 1 | Yes | 1.117 | 1.189 | 0.541 | 0.804–1.759 | 0.386 |
| Female age (y) at first cycle | 0.881 | <.001 | 0.554 | 1.008 | 0.778–0.879 | 0.098–0.968 | 0.701 |
| Female BMI (kg/m²) at first cycle | 1.011 | 0.554 | 1.008 | 0.968–0.049 | 0.701 |
| Male smoking history (%) | No | 1 | Yes | 0.900 | 0.979 | 0.662 | 0.586–1.637 | 0.937 |
| Varicocele history (%) | No | 1 | Yes | 1.963 | 1.991 | 1.32 | 0.788–5.033 | 0.145 |
| TMSC (10⁶ motile sperm) | 1.002 | 0.192 | 1.003 | 0.999–1.005 | 0.092 |
| DFI (%) categorized | ≤ 15 | 1 | 1.206 | 1.280 | 0.487 | 0.715–2.293 | 0.406 |
| | 15.1–20 | 1.306 | 1.519 | 0.474 | 0.676–3.408 | 0.312 |
| | 20.1–25 | 1.107 | 1.170 | 0.762 | 0.524–2.425 | 0.673 |
| | ≥ 30.1 | 0.782 | 0.751 | 0.390 | 0.389–1.451 | 0.394 |
| HDS (%) categorized | ≤ 7.5 | 1 | 1.160 | 1.376 | 0.539 | 0.815–2.322 | 0.233 |
| | 7.6–10 | 0.944 | 1.189 | 0.808 | 0.706–2.005 | 0.515 |
| | 10.1–15 | 1.051 | 1.191 | 0.832 | 0.686–2.067 | 0.534 |
| | ≥ 15.1 | 0.699 | 0.597 | <.001 | 0.499–0.723 | <.001 |

Note: BMI = body mass index; CI = confidence interval; DFI = DNA fragmentation index; HDS = high DNA stainability; ICSI = intracytoplasmic sperm injection; OR = odds ratio; TMSC = total motile sperm count.

Blachman-Braun. Pregnancy success after ICSI. Fertil Steril Rep 2020.

| TABLE 3 |

| Characteristic | ICSI attempts until pregnancy, median | 95% CI | P value |
| --- | --- | --- | --- |
| Overall | 2 | 1.84–2.16 | .597 |
| DFI (%) categorized | ≤ 15 | 2 | 1.81–2.19 | .632 |
| | 15.1–20 | 2 | 1.53–2.47 | .632 |
| | 20.1–25 | 2 | 1.51–2.49 | .632 |
| | 25.1–30 | 2 | 1.28–2.72 | .632 |
| | ≥ 30.1 | 2 | 1.27–2.73 | .632 |
| HDS (%) categorized | ≤ 7.5 | 2 | 1.72–2.28 | .632 |
| | 7.6–10 | 2 | 1.83–3.12 | .632 |
| | 10.1–15 | 2 | 1.64–2.36 | .632 |
| | ≥ 15.1 | 2 | 1.61–2.39 | .632 |

Note: CI = confidence interval; DFI = DNA fragmentation index; HDS = high DNA stainability; ICSI = intracytoplasmic sperm injection.

Blachman-Braun. Pregnancy success after ICSI. Fertil Steril Rep 2020.
A 15% was associated with a 5% increase in risk for early miscarriages, but there is conflicting data in other studies (13, 16).

Infertility and its associated treatment have a large economic burden (24). One IVF cycle in the United States can cost $10,000 to $15,000 (25). Therefore, it is imperative to identify superfluous tests that may increase cost and preclude couples from attempting IVF or repeat IVF. Our study reports that DFI and HDS values had no statistical significance in the number of cycles for IVF required to achieve pregnancy. With the high price of these tests, the dearth of value added may not justify their use (26).

To our knowledge, our study evaluating 1,050 cycles for ICSI is one of the largest series in the current literature, and is the largest series investigating the association between DFI as well as HDS and pregnancy success. Other strengths include that all ICSI treatments were performed in the same IVF center, and all DFI/HDS testing was done in the same commercial laboratory using SCSA, a commercially available standardized assay. Additionally, we presented demographic characteristics in both male and female partners that can affect pregnancy. Multiple studies have questioned the use of DFI and HDS as a predictor for pregnancy outcomes (13, 15, 16, 19, 22, 23, 27), however, few studies have evaluated the number of ICSI cycles needed to achieve pregnancy, which is valuable information that can be used to predict the cost-effect or financial investment once starting ICSI treatment and set couples expectations. Limitations of our study include its retrospective nature with a moderate sample size, no inclusion of the interval between cycles, no data on embryo quality, only analysis of the first pregnancy of couples regardless if couples achieved additional pregnancies with ICSI treatment, and consideration of only the first DFI and HDS results. Additionally, another limitation is that sperm DFI can change over time due to a multitude of reasons, and this may be the case in couples who underwent multiple rounds of ICSI. Further research is needed regarding DFI and HDS in IVF cycles and their association with embryo quality, incidence of miscarriage, recurrent IVF failure, and further pregnancy outcomes.

CONCLUSION

Our study showed that both DFI and HDS have limited utility as predictors of pregnancy achievement because the median number of ICSI attempts a couple undergo before achieving pregnancy was similar regardless the initial DFI or HDS. An increased female age and couples who required more ICSI attempts had lower chances of achieving pregnancy. Further studies are needed to confirm the true value of sperm DFI and HDS testing.

REFERENCES

1. Inhorn MC, Patrizio P. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. Hum Reprod Update 2015;21:411–26.
2. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: a review of literature. J Hum Reprod Sci 2015;8:191–6.
3. Sunderam S, Kissin DM, Crawford SB, Folger SG, Boulet SL, Warner L, et al. Assisted reproductive technology surveillance—United States, 2015. MMWR Surveill Summ 2018;67:1.
4. Masterson TA, Greer AB, Ramasamy R. Time to improvement in semen parameters after microsurgical varicocelectomy in men with severe oligospermia. Can Urol Assoc J 2019;13:E66–9.

5. Oehninger S, Ombelet W. Limits of current male fertility testing. Fertil Steril 2019;111:835–41.

6. Boulet SL, Mehta A, Kissin DM, Warner L, Kawwass JF, Jamieson DJ. Trends in use of and reproductive outcomes associated with intracytoplasmic sperm injection. JAMA 2015;313:255–63.

7. Dutta S, Majzoub A, Agarwal A. Oxidative stress and sperm function: a systematic review on evaluation and management. Arab J Urol 2019;17:87–97.

8. Baskaran S, Finelli R, Agarwal A, Henkel R. Reactive oxygen species in male reproduction: a boon or a bane? Andrologia 2020:e13577.

9. Wagner H, Cheng JW, Ko EY. Role of reactive oxygen species in male infertility: an updated review of literature. Arab J Urol 2017;16:35–43.

10. Kuchakulla M, Narasimman M, Khodamoradi K, Khosravizadeh Z, Ramasamy R. How defective spermatogenesis affects sperm DNA integrity. Andrologia 2020:e13615.

11. Castilla JA, Zamora S, Gonzalez MC, Luna Del Castillo JD, Roldan-Nofuentes JA, Clavero A, et al. Sperm chromatin structure assay and classical semen parameters: systematic review. Reprod Biomed Online 2010;20:114–24.

12. Cissen M, Wely MV, Scholten I, Marsell S, Bruin JP, Mol BW, et al. Measuring sperm DNA fragmentation and clinical outcomes of medically assisted reproduction: a systematic review and meta-analysis. PLoS One 2016;11:e0165125.

13. Evenson DP. The Sperm Chromatin Structure Assay (SCSA(R)) and other sperm DNA fragmentation tests for evaluation of sperm nuclear DNA integrity as related to fertility. Anim Reprod Sci 2016;199:56–75.

14. Stirrat GM. Recurrent miscarriage I: definition and epidemiology. Lancet 1990;336:673–5.

15. Jerre E, Bungum M, Evenson D, Giwercman A. Sperm chromatin structure assay high DNA stainability sperm as a marker of early miscarriage after intracytoplasmic sperm injection. Fertil Steril 2019;112:46–53.e2.

16. Speyer BE, Pizzey AR, Abramov B, Saab W, Doshi A, Sarna U, et al. Successful outcomes achieved in assisted reproduction cycles using sperm with high levels of high DNA stainability. Syst Biol Reprod Med 2015;61:293–9.

17. Zhang Z, Zhu LL, Jiang HS, Chen H, Chen Y, Dai YT. Predictors of pregnancy outcome for infertile couples attending IVF and ICSI programmes. Andrologia 2016;48:874–81.

18. Evenson DP. Sperm Chromatin Structure Assay (SCSA). Methods Mol Biol 2013;927:147–64.

19. Yang H, Li G, Jin H, Guo Y, Sun Y. The effect of sperm DNA fragmentation index on assisted reproductive technology outcomes and its relationship with semen parameters and lifestyle. Transl Androl Urol 2019;8:356–65.

20. Farhi J, Cohen K, Mizrahi Y, Weissman A, Raziel A, Orvieto R. Should ICSI be implemented during IVF to all advanced-age patients with non-male factor subfertility? Reprod Biol Endocrinol 2019;17:30.

21. Liang X, Mao Y, Wang Y, Liu S, Yan J. Female age affects the utility of sperm DNA fragmentation in predicting IVF and ICSI outcomes. Reprod Biomed Online 2019;39:955–62.

22. Oehninger S, Ombelet W. Limits of current male fertility testing. Fertil Steril 2019;111:835–41.

23. Pacey A. Is sperm DNA fragmentation a useful test that identifies a treatable cause of male infertility? Best Pract Res Clin Obstet Gynaecol 2018;53:11–9.

24. Borges E Jr, Zanetti BF, Setti AS, Braga D, Provenza MR, Laconelli A Jr. 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;112:483–90.

25. ESHRE Capri Workshop Group. Economic aspects of infertility care: a challenge for researchers and clinicians. Hum Reprod 2018;33:119–30.

26. IVF-Worldwide. The cost of IVF in different countries. Vol. 1. Research and Publication, 2008. Available at: https://ivf-worldwide.com/education/introduction/ivf-costs-worldwide/the-costs-of-ivf-in-different-countries.html, Accessed March 22, 2020.

27. Agarwal A, Cho C-L, Esteves SC, Majzoub A. The price and value of sperm DNA fragmentation tests. Transl Androl Urol 2017;6:5597–9.

28. Gat I, Li N, Yasovich N, Antes R, Kuznetsov V, Zohni K, et al. Sperm DNA fragmentation index does not correlate with blastocyst euploidy rate in egg donor cycles. Gynecol Endocrinol 2018;34:212–6.