Sperm chromatin structure assay high DNA stainability sperm as a marker of early miscarriage after intracytoplasmic sperm injection

Elsa Jerre, M.D.,[a] Mona Bungum, Ph.D.,[a] Donald Evenson, Ph.D.,[b] and Aleksander Giwercman, M.D., Ph.D.[a]

[a] Department of Translational Medicine, Lund University, Malmö, Sweden; [b] SCSA Diagnostics, Inc., Brookings, South Dakota

Objective: To determine whether high DNA stainability (HDS), as assessed by the sperm chromatin structure assay (SCSA), predicts the risk of early miscarriage after in vitro fertilization with intracytoplasmic sperm injection (IVF-ICSI).
Design: Retrospective cohort study of consecutive pregnancies after IVF and ICSI treatment.
Setting: Reproductive medicine center.
Patient(s): A total of 1,602 pregnancies after 832 IVF and 770 ICSI treatments.
Intervention(s): HDS measured using SCSA.
Main Outcome Measure(s): Early miscarriage ($\leq$12 weeks).
Result(s): The HDS represents the proportion of immature spermatozoa lacking the normal exchange of histone for protamine-complexed DNA, and the outcome parameter was early miscarriage ($\leq$12 weeks). For all treatments, the odds ratio (OR) and 95% confidence interval (CI) for early miscarriage was 1.41 (1.07–1.85) if HDS >15% compared with HDS $\leq$15%. When comparing the two HDS categories, for ICSI, the OR was 1.44 (1.01–2.04) whereas for IVF the results were not statistically significant.
Conclusion(s): There is a small but increased risk of early miscarriage if HDS >15% compared with HDS $\leq$15%. This increased risk is seen only after ICSI, not after IVF. These findings suggest that HDS can be used as a predictor of an increased risk of miscarriage in ICSI treatments. (Fertil Steril® 2019;112:46–53. ©2019 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: DNA fragmentation, early miscarriage rate, high DNA stainability, in vitro fertilization, sperm DNA integrity

Infertility is classified by the World Health Organization (WHO) as a disease that affects 15% to 20% of all couples trying to conceive (1). Assisted reproduction techniques (ART) are important tools for treating infertile couples, and the most efficient are in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (2). By now ART has resulted in over 6.5 million births worldwide (3). Because of delayed first pregnancies (4) and perhaps even declining sperm counts (5) it is likely that this number will continue to rise. Although the number of IVF and ICSI treatments has increased over the years, their "success rate"—the number of live births compared to the number of embryo transfers—has for the last 20 years remained less than 30% (6).

A substantial proportion of ART pregnancies are terminated by early miscarriage (before 12 weeks of gestation), and approximately 50% of those are believed to be related to chromosomal abnormalities, in particular aneuploidy (7–9). The factors known to increase the risk of miscarriage are infections, endocrine disturbances, and a suboptimal uterine environment as well as advanced maternal age (10, 11). Male factors such as age also

Received January 10, 2019; revised March 5, 2019; accepted March 8, 2019; published online April 28, 2019.
E.J. has nothing to disclose. M.B. has nothing to disclose. D.E. is the president and director of SCSA Diagnostics, which produces the SCSA test was used for this study. A.G. received grants from EU Interreg V, the Swedish Research Council, and the Swedish Governmental Fund for Clinical Research (ALF) during the conduct of the study, and personal fees from IBSA Pharmaceuticals, Sandoz, FINOX, and Ferring Pharmaceuticals outside the submitted work.
Supported by funds from the Swedish Research Council, Swedish Governmental Funding for Clinical Research and the EU Interreg V (ReproUnion).
Reprint requests: Aleksander Giwercman, M.D., Ph.D., Department of Translational Medicine, Lund University, Jan Waldenströms Gata 35, CRC-91-10-058, SE 21428 Malmö, Sweden (E-mail: aleksander.giwermcan@med.lu.se).

Fertility and Sterility® Vol. 112, No. 1, July 2019 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2019.03.013

affect the risk of miscarriage (12). Reducing the miscarriage rate is one of the strategies that might help in making ART more efficient and also reduce the problem of recurrent miscarriage in spontaneous pregnancies. To date, we lack reliable markers for the risk of early miscarriage.

Sperm DNA integrity can be assessed by several methods, one which being the sperm chromatin structure assay (SCSA) (13, 14). SCSA, a high-precision flow-cytometric test, is probably the most widely used of the clinical tests (15). It is the only sperm DNA fragmentation test that simultaneously measures both DNA strand breaks and chromatin structure.

For the SCSA, fresh or frozen/thawed semen samples are subjected to an acidic solution that denatures the DNA strands at sites of single- or double-strand breaks whereas the normal/native DNA stays intact (13). The sperm are then stained with the small molecule acridine orange (AO) that intercalates into the double-stranded DNA and emits a green fluorescence under 488 nm light. When AO binds to single-stranded DNA it collapses into a crystal that undergoes a metachromatic shift to red fluorescence (16). The DNA fragmentation index (DFI) is the ratio of red/red + green fluorescence and is the proportion of sperm in the semen sample that has measurable DNA strand breaks, as calculated by the dedicated software. The DFI has been shown to be a reliable predictor of male fertility potential for both in vivo and in vitro fertilization (17–21). Studies also have shown that a high DFI score is associated with an increased miscarriage rate (17, 21, 22).

From a clinical point of view, the predictive role of the other SCSA parameter, high DNA stainability (HDS), has been less investigated. The HDS parameter expresses the fraction of sperm with higher level of green fluorescence due to a lack of full exchange of histones for protamines; histone-complexed DNA is stained by AO to a higher degree than protamine-complexed DNA. Flow cytometric sorting shows that the HDS population has a more rounded morphology than normal sperm and lacks DNA strand breaks (14, 23, 24). It is believed to represent the immature sperm in the sample (14). Some studies suggest that semen samples with a high HDS level may lead to early embryo growth cessation (Booze, Brannian, and Evenson, 2018, unpublished data) as well as miscarriage, although the results are somewhat conflicting (18, 22, 25–29). Our study examined data from almost 5,000 IVF-ICSI cycles to investigate the degree to which the level of HDS is associated with the risk of early miscarriage in couples undergoing ART.

MATERIALS AND METHODS

Study Design and Population

Our retrospective study was based on data from all IVF-ICSI treatments performed at the Reproductive Medicine Centre, Skåne University Hospital in Malmö, Sweden, between August 2007 and September 2017. Altogether 8,163 cycles were performed. Of these, 1,581 cycles used frozen embryos from an earlier treatment and were excluded from the study. Another 44 cycles which used donated gametes and 1,632 cycles where no HDS level was recorded were also excluded. Additionally, 109 cycles were excluded because an ART method other than IVF or ICSI was used. Out of the remaining 4,797 cycles, a biochemical pregnancy was achieved in 1,627 cases, although an additional 25 cases were excluded from the study for being ectopic pregnancies or still ongoing at the time of this study. This resulted in 1,602 cycles for analysis, comprising 832 IVF and 770 ICSI treatments. The selection process is visualized in Supplemental Figure 1 (available online).

The research protocol was reviewed and approved by the ethics board in Lund (Dnr. 2015/006), and the couples signed a written informed consent before being included or were contacted by letter after treatment and offered an opt-out in case they did not wish to have their clinical data included in the analysis.

Semen Collection and Preparation

Semen samples were collected by masturbation on the day of ovum pickup. Standard sperm parameters were recorded according to World Health Organization guidelines (30). We froze 200 μL of raw semen at 80°C for later SCSA analysis. The remaining semen was prepared using a standard density gradient centrifugation method, PureSperm, 45% and 90% (Nidacon Ltd), to separate the motile sperm from lymphocytes, epithelial cells, senescent or immotile sperm, cell debris, bacteria, and seminal fluid.

Sperm Chromatin Structure Assay (SCSA)

The SCSA was performed on frozen/thawed semen that was diluted with Tris-NACL-EDT (TNE) buffer to a final concentration of 6×10^6 sperm/mL. This suspension was then treated with 400 μL of pH 1.20 buffer for 30 seconds to denature the DNA at the sites of strand breaks. Subsequently 1,200 μL of AO staining solution was added, and the sample was placed in a FACSCanto II (Becton Dickinson) flow cytometer. All samples were independently measured twice, and at least 5,000 sperm cells were analyzed per measurement. The flow cytometric data were analyzed using the dedicated software SCSA-Soft (SCSA Diagnostics). After every fifth sample analyzed, a reference sample was run to verify that the flow cytometer optics and fluidics remained precise. This reference sample came from a healthy donor obtained from the laboratory storage.

Oocyte Retrieval

Two types of protocol were used to achieve controlled ovarian stimulation; gonadotropin-releasing hormone (GnRH) antagonist short protocol or GnRH agonist long protocol. Orgalutran (MSD) or Cetrotide (Merck) was used in the antagonist protocol, and Synarela (Pfizer), Suprefact (Sanofi), or Suprecur (Sanofi) was used in the agonist protocol. Ovarian stimulation was performed with recombinant follicle-stimulating hormone (FSH) (Gonal-f; Merck), Puregon (MSD), Menopur (Ferring Pharmaceuticals), or Fostimon (NordicInfu Care), and the dosage was based on the woman's age, ovarian volume, baseline FSH level, and body mass index (BMI). Ovarian response was monitored by vaginal ultrasound to determine the count and size of the follicles. When two follicles had reached 17 mm an injection of human chorionic gonadotropin (hCG) (Ovitrelle, Merck; or Pregnyl, MSD) was given

TABLE 1

Demographic information of the study population divided by HDS level.

Characteristic	Total	HDS ≤7%	7< HDS ≤10%	10< HDS ≤15%	HDS >15%
Total ART (n)	1,602	501	362	357	382
IVF (n)	832	350	219	154	109
ICSI (n)	770	151	143	203	273
Female age (y, mean/SD)	32.3 (4.1)	32.8 (4.1)	32.4 (4.1)	32.3 (4.1)	31.6 (3.9)
Female BMI (mean/SD)	23.4 (3.2)	23.3 (3.1)	23.5 (3.3)	23.1 (3.1)	23.6 (3.1)
Previous IVF cycles (mean/SD)	0.70 (0.92)	0.72 (0.92)	0.65 (0.86)	0.74 (0.98)	0.70 (0.88)
Cycles with DFI >40%	46	15	8	9	14
Agonist protocol (n)	945	295	217	209	224
Antagonist protocol (n)	622	189	140	139	154
Unknown protocol (n)	35	17	5	9	4
Total FSH (in IU, median/IQR)	1,650 (1,012)	1,675 (1,125)	1,650 (990)	1,650 (900)	1,574 (934)
Embryo transfer					
On day 2	945	301	206	209	229
On day 3	356	94	89	86	87
On day 5	283	104	64	54	61
Aspirated oocytes (median/IQR)	10 (7)	9 (8)	10 (7)	10 (8)	11 (8)

Note: ART = assisted reproduction cycles; BMI = body mass index; DFI = DNA fragmentation index; FSH = follicle-stimulating hormone; HDS = high DNA stainability; ICSI = intracytoplasmic sperm injection; IQR = interquartile range; IVF = in vitro fertilization; SD = standard deviation.

Jerre. Sperm SCSA HDS and miscarriage risk. Fertil Steril 2019.

to induce final follicular maturation. Ovum pickup was conducted 35 hours after administration of hCG using a transvaginal ultrasound probe and needle (Cook Australia).

IVF and ICSI Procedures and Embryo Transfer

For IVF, sperm cells with a concentration of 150,000/mL were added to the oocytes. They were placed in an incubator with a gas phase of 6% CO_2, 5% O_2, and 89% N_2, in which fertilization took place during 90 minutes of coincubation. Before ICSI, the oocytes were denuded from cumulus cells using glass pipettes (SweMed Lab) and then rinsed. The injection was performed using ICSI pipettes (Pipette Company), and afterward the oocytes were placed in an incubator. The embryos with the best morphological features were chosen for embryo transfer on day 2, 3, or 5 after ovum pickup. The embryo transfers were performed using a Cook Soft 5000 catheter (Cook Australia), and 85% of the embryo transfers were single-embryo transfer.

Treatment Outcome

Biochemical pregnancy was defined as either a plasma hCG concentration >10 IU/L on day 12 after embryo transfer or a positive result on a commercially available urine pregnancy test on day 17. Early miscarriage was defined as the spontaneous expulsion of the gestational sac at gestational week ≤12, as verified by gynecologic ultrasound.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 25 software (SPSS Inc.). Primarily, using linear regression, the levels of HDS were compared between the groups (+) versus early miscarriage (−). This was done for the entire group as well as for ICSI and IVF, separately. Adjustments were made for the woman's age and BMI as well as DFI (DFI ≤40% or DFI >40%) (21).

Subsequently, treatments were categorized into four groups based on the quartiles for HDS: HDS ≤7% (reference group), 7< HDS ≤10%, 10< HDS ≤15% and HDS >15%. Subsequently, treatments were categorized into two groups: HDS ≤15% (reference group) and HDS >15%. Calculations were performed separately for IVF and ICSI as well as combined. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated using binary logistic regression analysis. The outcome was early miscarriage. All results were adjusted for female age, BMI, and DFI (DFI ≤40% or DFI >40%) (21). Post hoc power analysis showed that with $\alpha = 0.05$ and $\beta = 0.8$ the study was powered to show an unadjusted increase in miscarriage rate from 30% in HDS ≤15% to 38% in the HDS >15% group. $P \leq .05$ was considered statistically significant.

RESULTS

Demographic Data

The demographic data of the HDS groups are shown in Table 1, and the data of the treatment groups are shown in Table 2. There were no prominent differences between the HDS groups. The IVF group had lower DFI and HDS values as well as fewer previous ART treatments than the ICSI group. The mean HDS (± standard deviation [SD]) for IVF was 9.9% (±6.2% SD) and for ICSI 15% (±9.4% SD). The mean DFI was 13% (±7.3% SD) and 19% (±11% SD), respectively. The mean number of previous ART treatments was 0.47 for IVF and 0.96 for ICSI. Otherwise, no notable differences were seen between the treatment groups. Among the 490 miscarriages, 280 took place before week 6 and the remaining during the weeks 6–12.

HDS Levels in the (+) versus (−) Early Miscarriage Groups

For the entire group, the difference in HDS values was borderline statistically significantly higher in the (+) as compared to the (−) early miscarriage group (mean: 12.9% vs. 12.0%; 95% CI, −0.01; 1.8%; $P = .05$). This was also true for the ICSI group (mean: 14.7% vs. 13.3%; 95% CI, −0.14; 2.8%; $P = .075$), but no difference was seen for IVF treatments (mean: 7.0% vs. 7.1%; 95% CI, −1.1; 0.9%; $P = .09$).

TABLE 2

Demographic information of the study population divided by method of fertilization.

Characteristic	Total ART	IVF	ICSI
HDS (mean/SD)	12.3 (8.3)	9.9 (6.2)	15.0 (9.4)
DFI (mean/SD)	15.9 (9.7)	13.0 (7.26)	19.0 (11.0)
Female age (y, mean/SD)	32.3 (4.1)	32.6 (4.1)	32.0 (4.0)
Female BMI (mean/SD)	23.4 (3.2)	23.1 (3.1)	23.6 (3.2)
Previous ART cycles (mean/SD)	0.70 (0.92)	0.47 (0.76)	0.96 (0.99)
Agonist protocol (n)	945	496	449
Antagonist protocol (n)	622	320	302
Unknown protocol (n)	35	16	19
Total FSH in IU (median/IQR)	1,650 (1,012)	1,650 (1,020)	1,625 (943)
Embryo transfer			
On day 2	944	454	490
On day 3	356	181	175
On day 5	283	187	96
Aspirated oocytes (median/IQR)	10 (7)	10 (7)	10 (7)

Note: ART = assisted reproduction cycles; BMI = body mass index; DFI = DNA fragmentation index; FSH = follicle-stimulating hormone; HDS = high DNA stainability; ICSI = intracytoplasmic sperm injection; IQR = interquartile range; IVF = in vitro fertilization; SD = standard deviation.

Jerre. Sperm SCSA HDS and miscarriage risk. Fertil Steril 2019.

Early Miscarriage after IVF and ICSI Combined

The odds ratios for early miscarriage with HDS ≤7% as reference are shown in Table 3, and with HDS ≤15% as the reference in Table 4 and in Supplemental Figure 2 (available online). For HDS >15% compared with HDS ≤7%, a statistically significant increase in OR was seen (OR 1.45; 95% CI, 1.05–2.00; P=.022). The miscarriage rate of HDS ≤7% was 147 (29%) of 501 whereas it was 129 (34%) of 382 with HDS >15%. In comparison with HDS ≤7%, no statistically significant difference in OR was seen for 7< HDS ≤10% or 10< HDS ≤15%. For HDS >15%, compared with HDS ≤15%, the OR was statistically significantly increased (OR 1.41; 95% CI, 1.07–1.85; P=.014), and the miscarriage rates were 361 (30%) of 1,220 and 129 (34%) of 382, respectively.

Early Miscarriage after IVF

No statistically significant differences in ORs were seen with HDS ≤7% or with HDS ≤15% as the reference group. The odds ratios when comparing to HDS ≤15% are shown in Table 4 as well as in Supplemental Figure 2. The odds ratios for comparisons with HDS ≤7% are shown in Supplemental Table 1 (available online).

Early Miscarriage after ICSI

The odds ratios for early miscarriage when HDS ≤7% was used as the reference are shown in Supplemental Table 2 (available online), and the odds ratios when HDS ≤15% was used as the reference are shown in Table 4 and in Supplemental Figure 2.

For HDS >15% compared to HDS ≤7%, a statistically significant increase in OR was seen (OR 1.63; 95% CI, 1.01–2.62; P=.0046). The rate of miscarriage in the reference group was 50 (33%) of 151), and the rate for HDS >15% was 100 (37%) of 273). No statistically significant differences were seen for 7< HDS ≤10% or 10< HDS ≤15%, when compared with HDS ≤7%. Also when comparing HDS >15% to HDS ≤15%, a statistically significant increase was seen (OR 1.43; 95% CI, 1.01–2.03; P=.0045); the rates of miscarriage were 161 (32%) of 497 and 100 (37%) of 273, respectively.

DISCUSSION

In a study including almost 5,000 IVF-ICSI cycles resulting in more than 1,600 pregnancies, we found that HDS above the level of 15% was associated with an approximately 5% increase in risk of early miscarriage when using ICSI. For IVF patients no such increase was seen. Although this difference may not appear notable, when we take into consideration

TABLE 3

Impact of HDS on early miscarriage in IVF and ICSI combined.

HDS	n	Early miscarriage	OR (95% CI)	*P* value	AOR[a] (95% CI)	*P* value
HDS ≤7%	501	147 (29%)	Ref	—	Ref	—
7< HDS ≤10%	362	106 (29%)	1.00 (0.74–1.34)	.99	1.06 (0.77–1.47)	.72
10< HDS ≤15%	357	108 (30%)	1.05 (0.78–1.41)	.77	1.04 (0.75–1.44)	.82
HDS >15%	382	129 (34%)	1.23 (0.92–1.64)	.16	1.45 (1.05–2.00)	.022

Note: AOR = adjusted odds ratio; CI = confidence interval; HDS = high DNA stainability; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; OR = odds ratio; Ref = reference value.
[a] The AOR was obtained using female age, body mass index, and DNA fragmentation index as covariates. $P \leq .05$ was considered statistically significant.

Jerre. Sperm SCSA HDS and miscarriage risk. Fertil Steril 2019.

TABLE 4

Impact of HDS on early miscarriage.

HDS	n	Early miscarriage	OR (95% CI)	*P* value	AOR (95% CI)[a]	*P* value
Total ART						
HDS≤15%	1,220	361 (30%)	Ref	—	Ref	—
HDS>15%	382	129 (34%)	1.21 (0.95–1.55)	.12	1.41 (1.07–1.85)	.014
IVF						
HDS≤15%	723	200 (28%)	Ref	—	Ref	—
HDS>15%	109	29 (27%)	0.95 (0.60–1.49)	.82	1.15 (0.70–1.90)	.58
ICSI						
HDS≤15%	497	161 (32%)	Ref	—	Ref	—
HDS>15%	273	100 (37%)	1.21 (0.89–1.64)	.24	1.44 (1.01–2.04)	.043

Note: AOR = adjusted odds ratio; ART = assisted reproduction technology; CI = confidence interval; HDS = high DNA stainability; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; OR = odds ratio; Ref = reference value.

[a] The AOR was obtained using female age, body mass index, and DNA fragmentation index as covariates. $P \leq .05$ was considered statistically significant.

Jerre. Sperm SCSA HDS and miscarriage risk. Fertil Steril 2019.

that miscarriage is the outcome for a substantial number of pregnancies, our findings could have important clinical as well as biological implications.

Although previous studies have indicated an association between HDS and risk of miscarriage, our results can be considered as novel. Lin et al. (25) found that a high HDS was associated with an increased risk of early abortion in IVF but not in ICSI cycles whereas Speyer et al. (26) found a statistically significant, although small, negative correlation between HDS and the miscarriage rate after ICSI. However, both studies were based on a significantly lower number of treatment cycles, and Speyer et al. (26) included only couples with previous unsuccessful ART attempts and excluded couples with female-factor infertility. In our setup most treatments were single-embryo transfer whereas Speyer et al. (26) had double-embryo transfers as the standard procedure. Furthermore, Lin et al. (25) did not adjust for other factors that could cause miscarriage, and their definition of miscarriage differed from ours, as they only counted miscarriages after the sixth week of gestation.

Previous studies have shown that increased sperm DNA damage is associated with an increased risk of early miscarriage (31, 32) and that abnormal protamination is connected to DNA damage (33, 34). Yet high HDS is not associated with increased DNA damage (24), and in our calculations we have adjusted the results for the effect related to high DFI.

The biological background for increased HDS is not well understood. Wyrobek et al. (35) found that HDS is associated with frequency of aneuploidy in spermatozoa, but that has been the only study to investigate such an association in humans, although similar findings have been seen in bulls (36, 37). This is not surprising because immature sperm have an increased rate of aneuploidy and other chromosomal abnormalities (38–40). A higher proportion of immature spermatozoa (41, 42) as well as of aneuploid gametes (41, 43, 44) have been linked to an increased risk of miscarriage after ART. It can therefore be speculated that the increased risk of miscarriage seen in couples with HDS >15% might be due to high aneuploidy rate in spermatozoa, something we did not investigate in our study. Another plausible explanation is that HDS represents an immature population of spermatozoa that lack proper chromatin structure, which inhibits proper gene expression and leads to early embryo death and miscarriages (14).

It can be speculated why the impact of high HDS on the risk of early miscarriage was only seen in ICSI cycles. One plausible explanation might be the difference in the mode of fertilization. In IVF, due to the competition between spermatozoa, it is likely that mature spermatozoa have a higher chance of binding to the zona pellucida and penetrating the oocyte. In ICSI, on the other hand, when using samples with high HDS, there likely is an increased risk of an immature spermatozoa being injected into the oocyte, and those pregnancies are at a higher risk of miscarriage. In support of this hypothesis, Lathi and Milki (45) found that a higher proportion of early aborted embryos with aneuploidy was seen in ICSI pregnancies compared with those resulting from IVF. Additionally, Carrell et al. (46) found that patients treated with ICSI more often had aneuploid spermatozoa than those treated with IVF.

Our findings may have some biological as well as clinical implications. Elucidating the underlying biological mechanisms could help in understanding the causes of early miscarriage and thereby lead to developing preventive and therapeutic measures. In the clinical setup, the decision of whether to perform IVF or ICSI is often based on standard semen parameters after a gradient centrifugation. However, during a treatment course based on the outcome of earlier treatment cycles the couple may be offered a switch from IVF to ICSI. In this context, SCSA analysis may be additional tool in deciding the right treatment strategy. Whereas DFI above 20% may be indicative of a higher chance of live birth when using ICSI (18, 21), high HDS in cases of ICSI pregnancies terminated by early miscarriages might lead to considering standard IVF as alternative option. However, additional studies are needed to confirm our HDS data.

The main strength of our study is its large patient cohort, which was based on almost 500 miscarriages, in contrast with the two previous studies which were based on a total of 68 early abortions (25, 26). Owing to our large study population, it was possible to stratify the patients into subgroups and analyze the different methods of fertilization

separately. Another strength was that all treatments were performed in the same clinic and almost all were single-embryo transfer, thereby reducing the risk of bias due to differences in methodology or patient-selection criteria.

The main weakness of this study was its retrospective design. The patients were not randomized; instead, the method of fertilization was based on standard sperm parameters, patient history, and the outcome of earlier treatments. Furthermore, use of urine chemical pregnancy testing rather than ultrasound or even a blood test to determine the presence of a pregnancy might have led to some improper diagnoses of pregnancy. Additionally, the mean value for the time of insemination to documented miscarriage is not available.

CONCLUSION

In ICSI pregnancies we found an increased risk of early miscarriage for HDS >15% when compared with lower levels of HDS. No such association was found for IVF. This finding shows that HDS can be used as a predictor of early miscarriage in ICSI.

REFERENCES

1. World Health Organization. Infertility is a global public health issue. Available at: http://www.who.int/reproductivehealth/topics/infertility/perspective/en/.
2. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992;340:17–8.
3. Calhaz-Jorge C, De Geyter C, Kupka MS, de Mouzon J, Erb K, Mocanu E, et al. Assisted reproductive technology in Europe, 2013: results generated from European registers by ESHRE. Hum Reprod 2017;32:1957–73.
4. Swedish National Board of Health and Welfare. Statistics on pregnancies, births and newborns, 2016. Socialstyrelsen 2018. Available at: http://www.socialstyrelsen.se/publikationer2018/2018-1-6.
5. Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, et al. Temporal trends in sperm count: a systematic review and meta-regression analysis. Hum Reprod Update 2017;23:646–59.
6. Q-IVF. Årsrapport 2017 Resultat Trender Öppna jämförelser. Q-IVF Nationellt kvalitetsregister för assisterad befruktning; 2017, Report No. 10.
7. Stirrat GM. Recurrent miscarriage. Lancet 1990;336:673–5.
8. Stephenson MD, Awartani KA, Robinson WP. Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case-control study. Hum Reprod 2002;17:446–51.
9. Greenwold N, Jauniaux E. Collection of villous tissue under ultrasound guidance to improve the cytogenetic study of early pregnancy failure. Hum Reprod 2002;17:452–6.
10. Rai R, Regan L. Recurrent miscarriage. Lancet 2006;368:601–11.
11. Regan L, Rai R. Epidemiology and the medical causes of miscarriage. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14:839–54.
12. De la Rochebrochard E, Thonneau P. Paternal age and maternal age are risk factors for miscarriage; results of a multicentre European study. Hum Reprod 2002;17:1649–56.
13. Evenson DP, Darzynkiewicz Z, Melamed MR. Relation of mammalian sperm chromatin heterogeneity to fertility. Science 1980;210:1131–3.
14. Evenson DP, Larson KL, Jost LK. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 2002;23:25–43.
15. Björndahl L, Barratt CLR, Castilla JA, Menkveld R, Kvist U, editors. A practical guide to basic laboratory andrology. Cambridge: Cambridge University Press; 2010.
16. Darzynkiewicz Z, Traganos F, Sharpless T, Melamed MR. Thermal denaturation of DNA in situ as studied by acridine orange staining and automated cytofluorometry. Exp Cell Res 1975;90:411–28.
17. Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E, Purvis K, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod 1999;14:1039–49.
18. Bungum M, Humaidan P, Axmon A, Spano M, Bungum L, Erenpreiss J, et al. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod 2007;22:174–9.
19. Spano M, Bonde JP, Hjollund HI, Kolstad HA, Cordelli E, Leter G. Sperm chromatin damage impairs human fertility. Danish First Pregnancy Planner Study Team. Fertil Steril 2000;73:43–50.
20. Giwercman A, Lindstedt L, Larsson M, Bungum M, Spano M, Levine RJ, et al. Sperm chromatin structure assay as an independent predictor of fertility in vivo: a case-control study. IntJ Androl 2010;33:221–7.
21. Oleszczuk K, Giwercman A, Bungum M. Sperm chromatin structure assay in prediction of in vitro fertilization outcome. Andrology 2016;4:290–6.
22. Virro MR, Larson-Cook KL, Evenson DP. Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles. Fertil Steril 2004;81:1289–95.
23. Evenson D. The Sperm Chromatin Structure Assay (SCSA) and other sperm DNA fragmentation tests for evaluation of sperm nuclear DNA integrity as related to fertility. Anim Reprod Sci 2016;169:56–75.
24. Evenson DP. Sperm chromatin structure assay (SCSA): 30 years of experience with the SCSA. In: Zini A, Agarwal A, editors. Sperm chromatin: biological and clinical applications in male infertility and assisted reproduction. New York: Springer; 2011:125–49.
25. Lin MH, Kuo-Kuang Lee R, Li SH, Lu CH, Sun FJ, Hwu YM. Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. Fertil Steril 2008;90:352–9.
26. 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.
27. Saleh RA, Agarwal A, Nada EA, El-Tonsy MH, Sharma RK, Meyer A, et al. Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. Fertil Steril 2003;79(Suppl 3):1597–605.
28. Kazerooni T, Asadi N, Jadid L, Kazerooni M, Ghanadi A, Ghaffarpasand F, et al. Evaluation of sperm's chromatin quality with acridine orange test, chromomycin A3 and aniline blue staining in couples with unexplained recurrent abortion. J Assist Reprod Genet 2009;26:591–6.
29. Menezo Y, Clement P, Amar E. Evaluation of sperm DNA structure, fragmentation and decondensation: an essential tool in the assessment of male infertility. Translat Androl Urol 2017;6(Suppl 4):S553–6.
30. World Health Organization. Laboratory manual for the examination and processing of human semen. 5th ed. Geneva: WHO; 2010.
31. Zini A, Boman JM, Belzile E, Ciampi A. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod 2008;23:2663–8.
32. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S, et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Hum Reprod 2012;27:2908–17.
33. Aoki VW, Moskovtsev SI, Willis J, Liu L, Mullen JB, Carrell DT. DNA integrity is compromised in protamine-deficient human sperm. J Androl 2005;26:741–8.
34. Simon L, Castillo J, Oliva R, Lewis SE. Relationships between human sperm protamines, DNA damage and assisted reproduction outcomes. Reprod Biomed Online 2011;23:724–34.
35. Wyrobek AJ, Eskenazi B, Young S, Arnheim N, Tiemann-Boege I, Jabs EW, et al. Advancing age has differential effects on DNA damage, chromatin integrity, gene mutations, and aneuploidies in sperm. Proceed Nat Acad Sci USA 2006;103:9601–6.
36. Nagy S, Johannisson A, Wahlsten T, Ijas R, Andersson M, Rodriguez-Martinez H. Sperm chromatin structure and sperm morphology: their association with fertility in AI-dairy Ayrshire sires. Theriogenology 2013;79:1153–61.

37. Rybar R, Kopecka V, Prinosilova P, Kubickova S, Veznik Z, Rubes J. Fertile bull sperm aneuploidy and chromatin integrity in relationship to fertility. Int J Androl 2010;33:613–22.
38. Kovanci E, Kovacs T, Moretti E, Vigue L, Bray-Ward P, Ward DC, et al. FISH assessment of aneuploidy frequencies in mature and immature human spermatozoa classified by the absence or presence of cytoplasmic retention. Hum Reprod 2001;16:1209–17.
39. Benkhalifa M, Kahraman S, Biricik A, Serteyl S, Domez E, Kumtepe Y, et al. Cytogenetic abnormalities and the failure of development after round spermatid injections. Fertil Steril 2004;81:1283–8.
40. Lazaros L, Kaponis A, Vartholomatos G, Hatzi E, Botsari S, Plachouras N, et al. Using semen flow cytometry to evaluate association of ploidy status and chromatin condensation of spermatozoa with conventional semen parameters: clinical application in intrauterine insemination. Fertil Steril 2011; 95:110–5.
41. Lazaros LA, Vartholomatos GA, Hatzi EG, Kaponis AI, Makrydimas GV, Kalantaridou SN, et al. Assessment of sperm chromatin condensation and ploidy status using flow cytometry correlates to fertilization, embryo quality and pregnancy following in vitro fertilization. J Assist Reprod Genet 2011; 28:885–91.
42. Buffat C, Patrat C, Merlet F, Guibert J, Epelboin S, Thiounn N, et al. ICSI outcomes in obstructive azoospermia: influence of the origin of surgically retrieved spermatozoa and the cause of obstruction. Hum Reprod 2006; 21:1018–24.
43. Jia CW, Wang L, Lan YL, Song R, Zhou LY, Yu L, et al. Aneuploidy in Early Miscarriage and its Related Factors. Chin Med J 2015;128:2772–6.
44. Carrell DT, Wilcox AL, Lowy L, Peterson CM, Jones KP, Erickson L, et al. Elevated sperm chromosome aneuploidy and apoptosis in patients with unexplained recurrent pregnancy loss. Obstet Gynecol 2003;101:1229–35.
45. Lathi RB, Milki AA. Rate of aneuploidy in miscarriages following in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril 2004;81:1270–2.
46. Carrell DT, Emery BR, Wilcox AL, Campbell B, Erickson L, Hatasaka HH, et al. Sperm chromosome aneuploidy as related to male factor infertility and some ultrastructure defects. Arch Androl 2004;50:181–5.

La alta capacidad de tinción del ADN del espermatozoide analizada por el ensayo de la estructura de la cromatina del espermatozoide como marcador de aborto espontáneo temprano tras la inyección intracitoplasmática de espermatozoides

Objetivo: Determinar si la alta capacidad de tinción del ADN (HDS), según lo evaluado por el ensayo de la estructura de la cromatina del espermatozoide (SCSA), predice el riesgo de aborto espontáneo temprano tras la fecundación in vitro mediante inyección intracitoplasmática de espermatozoide (FIV-ICSI).

Diseño: Estudio retrospectivo de cohorte de embarazos consecutivos después de un tratamiento de FIV e ICSI.

Entorno: Centro de medicina reproductiva.

Paciente (s): Un total de 1,602 embarazos después de 832 tratamientos de FIV y 770 de ICSI.

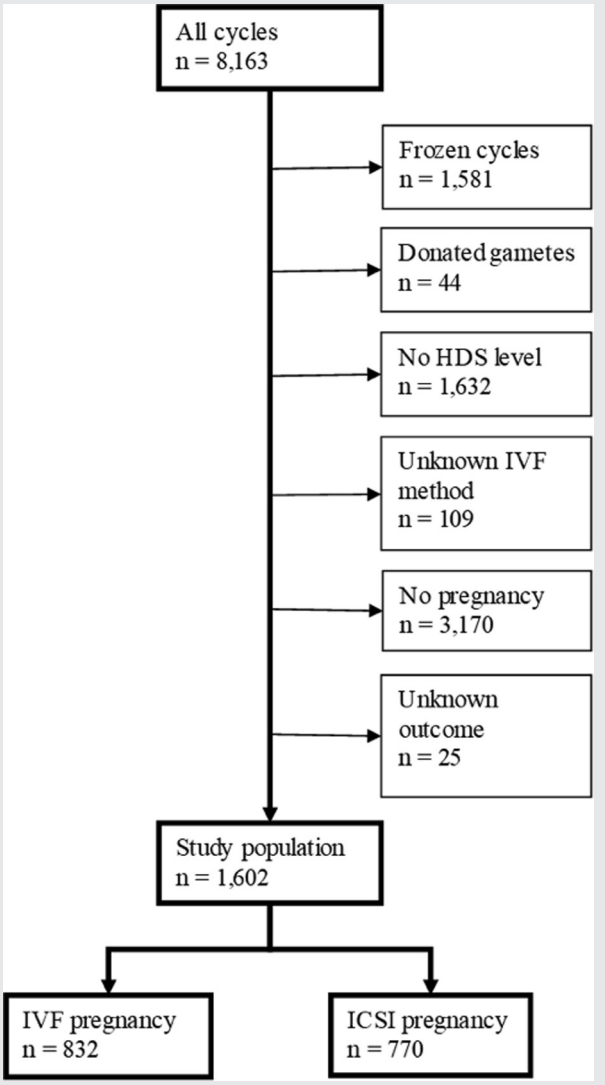
Intervención (es): HDS medido utilizando SCSA.

Principales medidas de resultado: Aborto espontáneo temprano ($\leq$ 12 semanas).

Resultado (s): El HDS representa la proporción de espermatozoides inmaduros que carecen del intercambio normal de histona por el complejo-protamina en el ADN, y el parámetro de resultado fue el aborto espontáneo temprano ($\leq$ 12 semanas). Para todos los tratamientos, la proporción de probabilidades (OR) con el intervalo de confianza (IC) del 95% para el aborto espontáneo temprano fue de 1.41 (1.07-1.85) si HDS $>$ 15% comparado con HDS $\leq$ 15%. Al comparar las dos categorías de HDS, para ICSI, el OR fue de 1.44 (1.01-2.04) mientras que para la FIV los resultados no fueron estadísticamente significativos.

Conclusión (es): Hay un pequeño pero incrementado riesgo de aborto espontáneo si HDS es $>$ 15% en comparación con HDS $\leq$ 15%. Este aumento del riesgo se observa solo después de la ICSI, no después de la FIV. Estos hallazgos sugieren que la HDS se puede usar como un predictor de un mayor riesgo de aborto espontáneo en los tratamientos con ICSI.

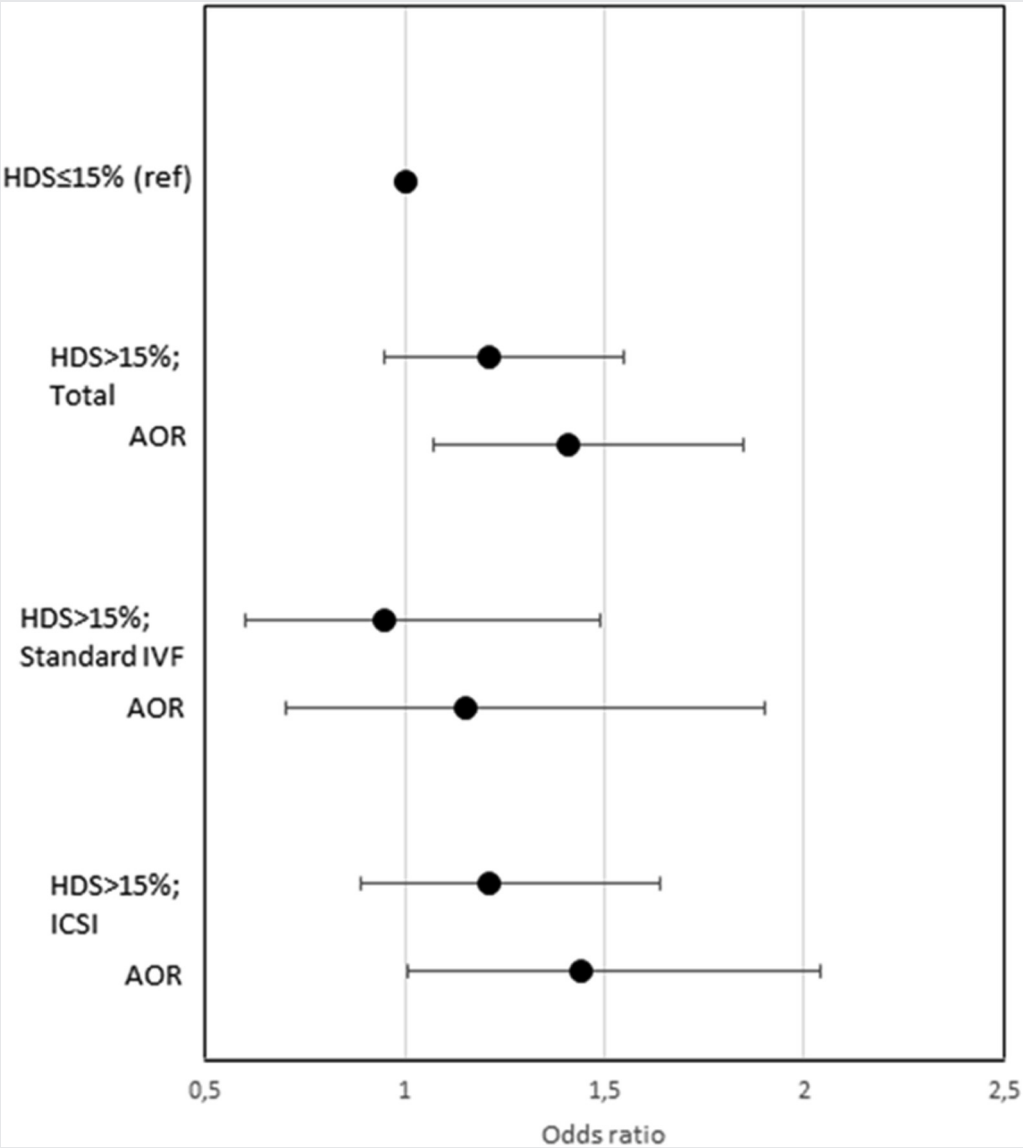
SUPPLEMENTAL FIGURE 1



Flow chart illustrating selection of study population.

Jerre. Sperm SCSA HDS and miscarriage risk. Fertil Steril 2019.

SUPPLEMENTAL FIGURE 2



Odds ratios for early miscarriage for HDS >15% compared with HDS ≤15% for IVF and ICSI separately and for all treatments (total). Unadjusted and adjusted for female age, body mass index, and DNA fragmentation index (AOR). HDS = high DNA stainability.

Jerre. Sperm SCSA HDS and miscarriage risk. Fertil Steril 2019.