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human reproduction
ORIGINAL ARTICLE ***Infertility***

Novel use of COMET parameters of sperm DNA damage may increase its utility to diagnose male infertility and predict live births following both IVF and ICSI

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STUDY QUESTION: Do the Comet parameters of the proportions of sperm with low or high DNA damage improve the power of the test in the diagnosis of male infertility and/or prediction of IVF and ICSI live birth rates?

SUMMARY ANSWER: The mean Comet score and the scores for proportions of sperm with high or low DNA damage were useful in diagnosing male infertility and provided additional discriminatory information for the prediction of both IVF and ICSI live births.

WHAT IS KNOWN ALREADY: Sperm DNA damage impacts adversely on male fertility and IVF outcomes.

STUDY DESIGN, SIZE, DURATION: A retrospective study was performed involving a total of 457 participants (381 patients and 76 fertile donors). Data was collected from a fertility clinic between 2015 and 2017.

PARTICIPANTS/MATERIALS, SETTING, METHODS: A total of 381 consecutive male partners of couples attending for ART and 76 fertile donors were included in the study. DNA fragmentation was measured by the alkaline Comet assay. Receiver operator characteristic curve analysis (area under the ROC curve (AUC)) was used to determine the value of average Comet score (ACS), low Comet score (LCS) and high Comet score (HCS) to diagnose male factor infertility. In total, 77 IVF and 226 ICSI cycles were included to determine thresholds for each parameter (AUC analysis) and to compare live birth rates (LBRs) following each ART.

MAIN RESULTS AND THE ROLE OF CHANCE: ACS, HCS and LCS were predictive of male infertility ($AUC > 0.9$, $P < 0.0001$). IVF LBRs declined once DNA damage exceeded the threshold levels. HCS showed the sharpest decline. Following ICSI, the highest LBRs were in men whose DNA damage levels approached the fertile range. Trends differed in IVF. LBRs decreased as damage increased whereas in ICSI the LBRs decreased but then remained stable.

LIMITATIONS, REASONS FOR CAUTION: Since this is the first study to show the impact of sperm DNA damage on ICSI live births, a prospective study should be performed (stratifying patients to IVF or ICSI based on these thresholds) to validate this study.

WIDER IMPLICATIONS OF THE FINDINGS: Our study presents novel information towards elucidating the genetic basis of male infertility and secondly on relevance of the extent of DNA damage as an impending factor in both IVF and ICSI success.

STUDY FUNDING/COMPETING INTEREST(S): This study was supported by Examenlab Ltd, The Lister Clinic, Cryos International and Imperial College London NHS Trust. No external funding was obtained for this study. SL and KL are employees of Examenlab Ltd, a university spin-out company with a commercial interest in sperm DNA damage. No other author has a conflict of interest to declare.

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Key words: ART / Comet / DNA fragmentation / IVF/ICSI outcomes / male infertility / live births / threshold values

Introduction

Male infertility has become the most common cause of a couple's difficulties in conceiving a child and is reported to be the most common cause of fertility cycles being carried out (Human Fertility Authority, UK; Fertility treatment 2014–2016 Trends and figures, 2018). Yet, most men presenting with infertility are not thoroughly investigated before they embark on infertility treatment. This may result in overtreatment of the infertile couple with IVF/ICSI, leading to psychological and physical burdens. This also has significant financial impacts on couples' and health care economies.

Sperm DNA damage has emerged as a robust biomarker of male fertility and can be increased by a number of factors including hormonal anomalies (Lu et al., 2018), varicoceles (Li et al., 2012; Tatem and Brannigan, 2017) or chronic infection (La Vignera et al., 2011). DNA damage is often related to excessive production of reactive oxygen species (ROS) and therapeutic interventions (e.g. varicocele treatment) have the potential to reduce ROS and improve sperm DNA integrity (Showell et al., 2012). It has been suggested that sperm DNA quality assessments are powerful tools for male fertility assessment including those diagnosed with idiopathic infertility (Simon et al., 2014; Osman et al., 2015).

Sperm DNA tests to date correlate with IVF but not with ICSI outcomes. This reduces their usefulness, as ICSI continues to dominate ART in most countries. There are several commonly used tests: terminal deoxynucleotidyl transferase (TdT) dUTP nick end labeling (TUNEL), the sperm chromatin structure assay (SCSA), sperm chromatin dispersion (SCD) or Halo test and the single-cell gel electrophoresis assay also known as the Comet used in this study under alkaline pH conditions (Kindzelskii et al., 2002; Robinson et al., 2012; Simon et al., 2014) measuring different aspects of damage and using different thresholds. The Comet and TUNEL measure DNA strand breaks and are associated with all the fertility checkpoints from fertilization to embryo quality, clinical pregnancy and miscarriage as well as live birth. The other tests (SCSA and Halo) measure chromatin compaction and do not correlate with live births (Robinson et al., 2012; Simon et al., 2014; Cissen et al., 2016).

The Comet assay quantifies the level of DNA damage in individual sperm and therefore can be used to determine the degree of heterogeneity of DNA quality in a whole sperm population.

The aims of this study were to quantify low, high and average damage levels throughout semen samples from fertile donors and compare them with those of men attending for fertility treatment. Secondly, we assessed the diagnostic and predictive power of these novel Comet parameters in IVF and ICSI.

Materials and Methods

Study populations

There were two separate study populations in this study.

The first population was fertile donors and included 76 men. The inclusion criteria were time to pregnancy less than 12 months of unprotected intercourse; healthy; normal semen according to the World Health Organization (WHO) (2010); no hereditary diseases; seronegative for HIV, syphilis, hepatitis B and C, herpes and cytomegalovirus; and no bacterial infections. All men were aged 18–45 years in accordance with Danish law.

The second population was men from infertile couples and included 381 men attending for ART. The inclusion criteria were minimum of 1-year unprotected intercourse without pregnancy and no genetic abnormalities leading to male infertility other than anomalies in semen analysis.

The different Comet scores of these patients were compared with ART outcomes. A subgroup of 166 men presenting with infertility but with normal semen parameters were assessed separately.

Sperm DNA damage assessment

Semen samples were collected by masturbation after 2–5 days of abstinence and on the day of ART treatment. Semen was analysed according to the WHO (2010) manual. Sperm DNA fragmentation was assessed using single-cell gel electrophoresis (alkaline Comet) assay, previously modified for human sperm (Hughes et al., 1997; Donnelly et al., 1999). Briefly, aliquots of native semen were adjusted using phosphate-buffered saline (PBS) to give a sperm concentration of 6×10^6 mL^{-1}. Following this initial preparation, membrane lysis, protamine and histone removal, electrophoresis, ethidium bromide staining and Comet scoring (Komet 7.0, Andor Technologies, Belfast, UK) were employed to analyse 50 sperm per slide, in duplicate. All steps were carried out in a temperature- and humidity-controlled environment to prevent induced DNA damage. Our previous study reported an intra-assay coefficient variation of 6% for this assay (Hughes et al., 1997).

In this study, the samples from fertile men were cryopreserved using a cryoprotectant supplemented, fast-freezing protocol. In a validation study of our protocol using plunge freezing without any cryoprotectant, we observed $30.1 \pm 4.6\%$ (mean $\pm$ SEM) DNA damage in fresh semen from a range of fertile and infertile men compared with $34.3 \pm 5\%$ DNA damage in freeze-thawed semen ($P = 0.13$ where $n = 157$; unpublished data).

Female assessment and treatment pre-ART

Ovarian stimulation was performed using either an agonist or antagonist protocol (clinician choice) and determined by female age, previous response and markers of ovarian reserve. Standard IVF/ICSI protocols were used as per unit guidelines.

Prior reproductive histories

The couples in the study had a range of prior unsuccessful reproductive outcomes from a variety of fertility clinics, varying from ectopic pregnancies, preclinical miscarriages and clinical miscarriages, failing to reach an embryo transfer to the most common reason: failed implantation.

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Table I The evaluation of novel sperm DNA fragmentation parameters in male infertility diagnosis.

Result parameter	Threshold value	AUC curve (95% CI)	*P* value	Sensitivity	Specificity	PPV	NPV	Odds Ratio
ACS	≥26%	**0.925** (0.893–0.956)	<0.0001	0.735	1.000	1.000	0.632	–
LCS	≤74%	**0.936** (0.908–0.964)	<0.0001	0.783	0.934	0.963	0.664	51.2 (19.3–136.5)
HCS	≥4%	**0.909** (0.872–0.942)	<0.0001	0.843	0.803	0.903	0.701	21.9 (10.8–44.3)

The AUC curve, sensitivity and specification, and positive and negative predictive values (PPV, NPV) are indications of the strength of sperm DNA damage as a diagnostic tool for male infertility They are based on comparisons of average Comet score (ACS), low Comet score (LCS) and high Comet score (HCS) of 76 fertile men and 184 men with normozoospermia presenting for fertility investigations. Threshold values were determined from the AUC curve by maximising the sum of sensitivity and specificity.

Statistical Analysis

Data were analysed using the statistical software XLSTAT-Biomed (Addinsoft, New York, NY, USA). Study variables were ages of female and male participants, the three Comet parameters in native semen (as a percentage of total population of sperm assessed) and IVF and ICSI outcomes as pregnancies and live birth rates. Threshold values were determined from the area under the ROC curve (AUC) by maximising the sum of sensitivity and specificity.

Chi-squared tests and logistic regression were used. In addition, Fisher's exact test was used to analyse some data due to the small number of live births.

Distribution of sperm DNA damage across a semen sample in fertile compared with infertile men

The conventional average Comet score (ACS; mean of all Comets scored) and two novel parameters, namely the low Comet score (LCS: percentage sperm with a low Comet score) and the high Comet score (HCS: percentage sperm with a high Comet score), were determined for fertile donors and compared with men attending for infertility investigations.

Evaluation of Comet parameters as a diagnostic marker for male infertility

To evaluate the ability of each Comet parameter to diagnose male infertility, 76 sperm donors (kindly provided by CRYOS, Aarhus, Denmark) and 184 men with normozoospermia were compared using the AUC, and thresholds determined.

Evaluation of Comet parameters as a prognostic marker for IVF and ICSI

To evaluate the ability of each Comet parameter to predict a live birth following IVF and ICSI, 77 IVF and 226 ICSI cycles were analysed using the AUC, and thresholds determined in the same way as for male infertility.

Relationship between Comet parameters and ART outcomes

Comet profiles, within the live birth and no birth groups for IVF and ICSI, were compared by calculating the mean, SE and SD. Since numbers of live births were small, the chi-squared test was used to assess whether there was a significant difference between the number of live births above and below each Comet threshold for both IVF and ICSI. A probability value of less than 0.05 was regarded as significant.

Results

Comparison of the novel LCS and HCS Comet parameters in fertile and infertile men

Seventy-six recently proven fertile sperm donors and 184 male partners of couples presenting with infertility were compared (Table I). Differences in ACS, LCS and HCS were significant ($P < 0.0001$). To maximise the utility of data available, the proportions of HCS and LCS were calculated to assess their clinical value in comparison with ACS. The ACS, LCS and HCS of fertile donors and men presenting for infertility treatment were compared graphically (Fig. 1a and b). The fertile male distribution demonstrated a marked skew to the left indicating that the majority of sperm had good-quality DNA. In this example, this was calculated as 90% of their sperm. In contrast, the infertile males had a distribution of sperm DNA with a skew to the right, indicating that only 56% of sperm had good-quality DNA, with the rest of their sperm having medium to high DNA damage.

Diagnostic value in predicting male infertility

We calculated the AUCs, positive and negative predictive values (PPV, NPV) and sensitivity and specificity for each parameter (Table I). All were highly significant ($P < 0.0001$). The OR was highest using LCS, and HCS were highly significant. PPVs give the number of subjects who have abnormal sperm DNA damage as classified correctly as infertile, and conversely, the NPV shows the number of subjects with DNA damage below our given thresholds as classified as fertile. PPV and NPV were similarly robust with all parameters.

Effect of sperm DNA damage compared with female age on live birth outcomes after IVF

Men were divided into groups using threshold values (ACS $\leq$ 29%, LCS $\geq$ 64%, HCS $\leq$ 6%) and their live birth rates (LBRs) after IVF cal-

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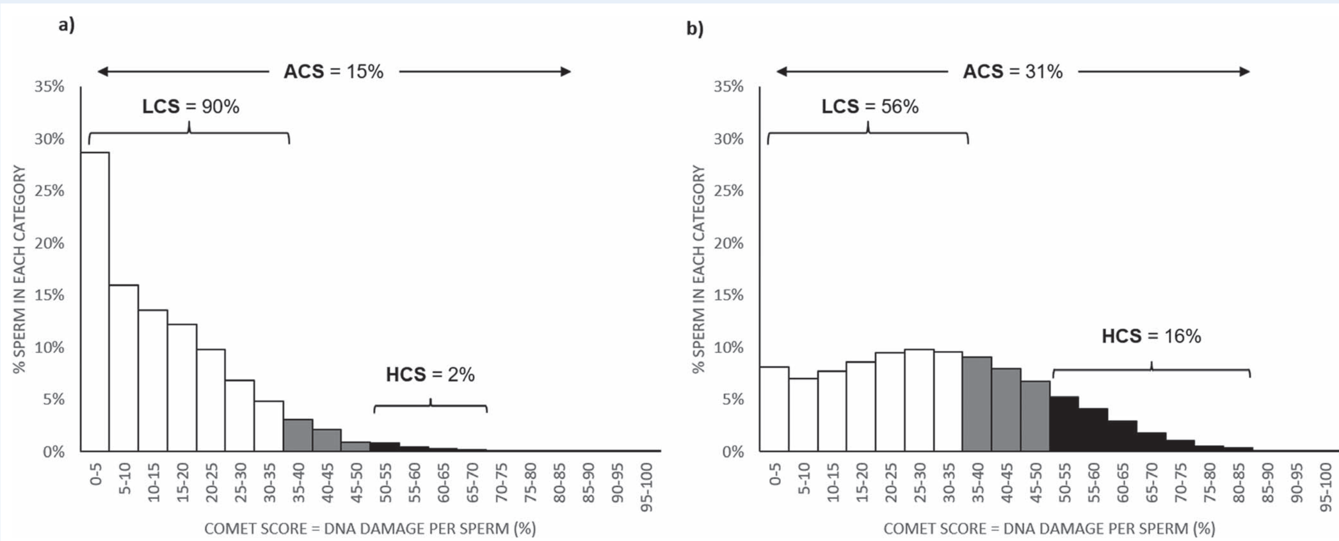

Figure 1 Sperm Comet plots for fertile men compared with men from couples with idiopathic infertility. (a) Fertile men A graphical depiction of the range of DNA damage in sperm; grouped in percentiles, from 76 fertile donors with normal semen profiles. ACS: the average DNA damage per sperm in the sample. Low DNA damage (%; LCS)—the proportion of sperm in the sample with good DNA needed for a successful pregnancy. High DNA damage (%; HCS)—the proportion of sperm in the sample with poor DNA unlikely to lead to a successful natural or ART pregnancy. **(b)** Infertile men A graphical depiction of the range of DNA damage in sperm, grouped in percentiles from 166 men with normal semen profiles but from couples with idiopathic infertility.

culated. LBRs were significantly lower in the group with DNA damage, whether assessed by ACS, LCS or HCS (Fig. 2, Table II).

Logistic regression was used to evaluate the relative significance of different possible contributory factors on LBR including ACS, LCS, HCS, male age, female age and anti-Müllerian hormone (AMH) plasma levels (Table III). Male age, ACS ≤ 29% and female AMH were applied in a stepwise backwards logistical regression but were rejected as statistically non-significant. HCS was the most significant measure of DNA damage with an OR of 7.03 (95% CI 1.76–28.14). Female age was significant but with a much lower OR of 0.82 (95% CI 0.68–0.98). The odds for female age were reduced by 18%, compounded year on year. In the IVF group, 41 of the 77 (53%) women were 38–45 years old. The mean age for men was 40.1 (SD 0.6.) years. The wide CIs were a function of the small numbers in the IVF group.

Effect of sperm DNA damage compared with female age on live birth outcomes after ICSI.

Men were divided into two groups using the threshold values (ACS ≤ 27%, LCS ≥ 68%, HCS ≤ 10%), and their LBRs after ICSI were calculated. LBRs were significantly lower in the group with DNA damage, whether assessed by ACS, LCS or HCS (Fig. 3, Table IV).

When we applied logistic regression (Table V) as above, we found that again HCS and female age were the most significant factors. However, in ICSI, increasing female age reduced the chance of a live birth less than in IVF. The OR for female age was 0.88 for women of whom 97 out of 226 (43%) were 38–45 years. The mean age for men was 39.6 (SD 0.4) years. Here, the odds were only reduced by 12%, compounded year on year. However, if HCS was low (≤10%), the odds in favour of a live birth were still multiplied by ~2—significant, although less than in IVF.

Comparison of pregnancy and LBR following IVF and ICSI categorised by ranges of sperm DNA fragmentation

In 77 couples having IVF, pregnancies and live births were divided into groups by sperm DNA fragmentation ranges (ACS: 0–15; 16–29, 30–45, >45%, data not shown). The major decline in success occurred when ACS exceeded by 29%. In 226 couples having ICSI, pregnancies and live births were again divided into groups by sperm DNA ranges (ACS: 0–15; 16–27, 28–45, >45%). Both pregnancy and live births were markedly higher (both were 50%) when ACS was less than 15%. Live births decreased to 43% as sperm DNA damage increased between 15 and 27% and further to 30% when ACS was 28–45%. Thereafter, the success rates remained constant (data not shown).

Simple theoretical model to predict live births for men with idiopathic infertility who were offered ICSI rather than IVF following a diagnosis of high ACS

In a typical group of men diagnosed with idiopathic infertility with normozoospermic semen analyses, the first route of treatment would be IVF. From the 184 men in this category, we calculated that their cycles would result in 32 births (17.4%). However, if those couples, whose lack of success was associated with high sperm DNA fragmentation (i.e. ACS ≥ 29%), were treated instead by ICSI, those cycles would have led to an increase of 24 more live, taking the ICSI total to 56 births (30%; Fig. 4).

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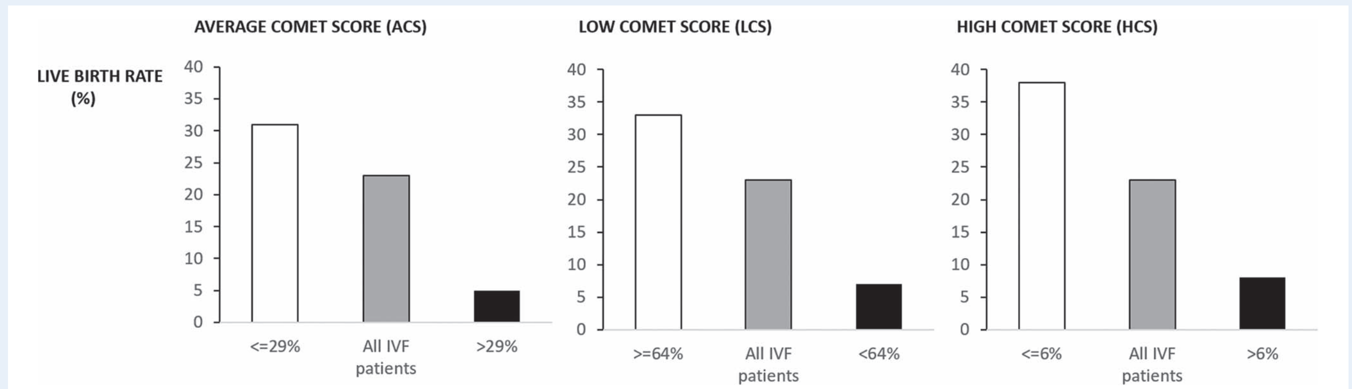

Figure 2 **IVF live birth rates above and below thresholds.** IVF live birth rates divided into groups above and below ACS, HCS and LCS thresholds and also combined for all patients.

Table II **The effects of novel sperm Comet parameters on live births after IVF.**

	DNA damage					
N **= 77**	**ACS**		**HCS**		**LCS**	
Threshold (%)	≤29	>29	≤6	>6	≥64	<64
Cycles (*N*)	55	22	40	37	49	28
Live births (*N*)	17	1	15	3	16	2
Live births (%)	31	0	37	1	33	0
No births	38	21	25	34	33	26
P value		**0.016**		**0.003**		**0.012**

Values are actual numbers of cycles or live births split into groups above or below the threshold for.
ACS, HCS or LCS using a total of 77 IVF cycles. Values were calculated using Fisher's exact test as numbers were low.

Table III **Logistic regression analysis of individual predictors of IVF live births.**

							95% CI for Exp β	
Predictor	β	**SE** β	**Wald's** χ^2	**df**	***P***	**Exp** β	**Lower**	**Upper**
Female age	−0.203	0.093	4.73	1	0.030	0.82	0.68	0.98
HCS ≤6%	1.95	0.707	7.60	1	0.006	7.03	1.76	28.14

Logistic regression was used to evaluate the relative significance of different possible contributory factors on live birth including ACS, LCS, HCS, male age, female age and anti-Müllerian hormone (AMH). Female age and HCS were significant ($P < 0.05$). Male age, ACS ≤29% and female AMH were applied in a stepwise backwards logistical regression but were rejected as statistically non-significant. $N = 77$.

Discussion

The importance of this study is 2-fold: first, it may reduce overtreatment of patients by ART and, second, improve the success of ART by selecting an evidence-based choice of IVF or ICSI.

Semen analysis is a descriptive evaluation, which is unable to discriminate between the sperm of fertile and infertile men (Lewis and Kumar, 2015). The fifth edition of the WHO Manual (World Health Organization, 2010) shows a reduction in the 'normal' range, which may be a retrograde step, with 15% of men previously classified as subfertile now being classified in the normal range and investigated no further (Pasqualotto *et al*., 2000). The usefulness of a semen analysis to predict ART outcomes is limited in IVF (Simon *et al*., 2013) and appears to be of no value in ICSI.

Many genomic studies have been performed over the past 20 years, and two important issues have emerged. First, there is consensus that men in infertile couples have more sperm DNA damage. Second, this damage points to a potentially trans-generationally hazardous cause of male infertility with adverse consequences for offspring (Simon *et al*., 2013; Lewis and Kumar 2015).

The commonly used tests for DNA damage are the SCSA, TUNEL, Halo and Comet assays. The TUNEL is a direct test for strand breakpoints. In the TUNEL, a TdT label, the DNA strand breaks with fluorescent dUTP nucleotides. There are many protocols for the TUNEL,

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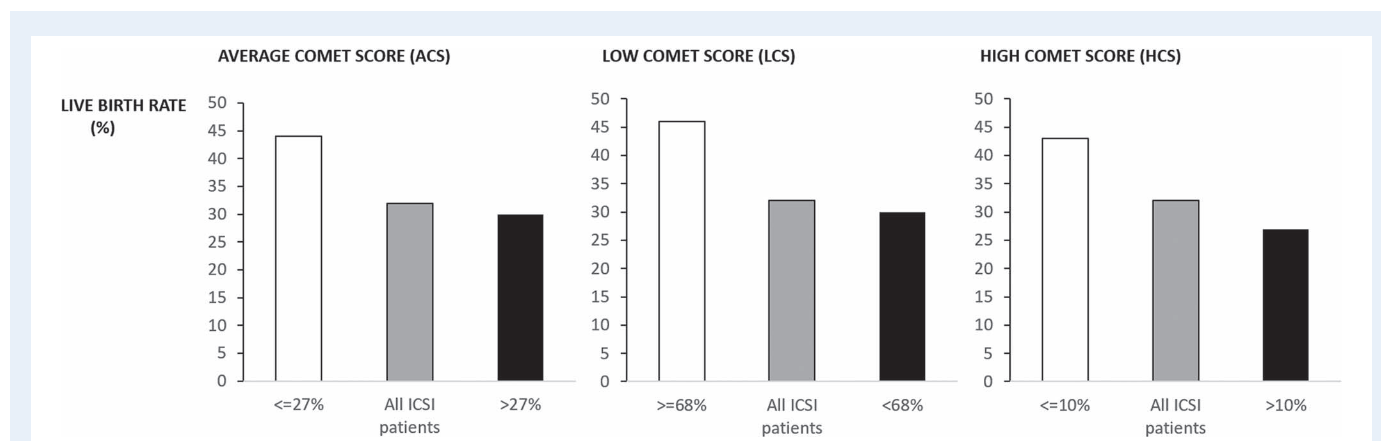

Figure 3 ICSI live birth rates above and below thresholds. ICSI live birth rates divided into groups above and below ACS, HCS and LCS thresholds and combined for all patients.

Table IV The effects of novel sperm Comet parameters on live births after ICSI.

	DNA damage					
N **= 226**	**ACS**		**HCS**		**LCS**	
%	≤27	>27	≤10	>10	≥68	<68
Cycles	50	176	81	145	41	185
Live births	22	52	35	39	19	55
Live births (%)	44	29	43	27	46	30
No births	28	124	46	106	22	130
P value		**0.062**		**0.018**		**0.045**

Values are actual numbers of cycles or live births split into groups above or below the threshold for ACS, HCS or LCS using a total of 226 ICSI cycles.
P values were calculated using Fisher's exact test as numbers were low.

Table V Logistic regression analysis of individual predictors of ICSI live births.

Predictor	β	SE β	Wald's χ^2	df	*P*	Exp β	95% CI for Exp β Lower	95% CI for Exp β Upper
Female age	−0.131	0.035	14.04	1	<0.001	0.88	0.82	0.94
HCS ≤ 10%	0.664	0.303	4.80	1	0.028	1.94	1.07	3.52

Logistic regression was used to evaluate the relative significance of different possible contributory factors on live birth including ACS, LCS, HCS, male age, female age and AMH. Female age and HCS were significant ($P < 0.05$). Male age, ACS ≤ 29% and female AMH were applied in a stepwise backwards logistical regression but were rejected as statistically non-significant. $N = 226$.

some with inadequate DNA decondensation steps (De Iuliis *et al.*, 2009). Studies using it cannot be easily compared. The biggest criticism has been the absence of clinical thresholds for diagnosis and treatment outcomes. In the Comet assay, lysis and decondensation steps have removed plasma membranes and associated histones and protamines so the Comet tail consists only of broken strands of DNA that are then measurable by fluorescent staining as they migrate in an electrophoretic field.

Cryopreservation can impair sperm motility and morphology (Liu *et al.*, 2016). In contrast, one of the benefits of the unique tightly coiled structure of sperm DNA is that it is resistant to cryoinjury, with the majority of studies showing an absence of deleterious effects on DNA. This is supported by the widespread global use of frozen donor sperm leading to healthy live births (Duru *et al.*, 2001; Duty *et al.*, 2002; Isachenko *et al.*, 2004; Punyatanasakchai *et al.*, 2008; Steele *et al.*, 2000). Furthermore, freeze-thawed sperm from infertile men has also been used extensively and successfully in artificial insemination and IVF programmes.

The alkaline Comet is a single-cell gel electrophoretic assay that quantifies broken strands of DNA in individual sperm. Its major benefit

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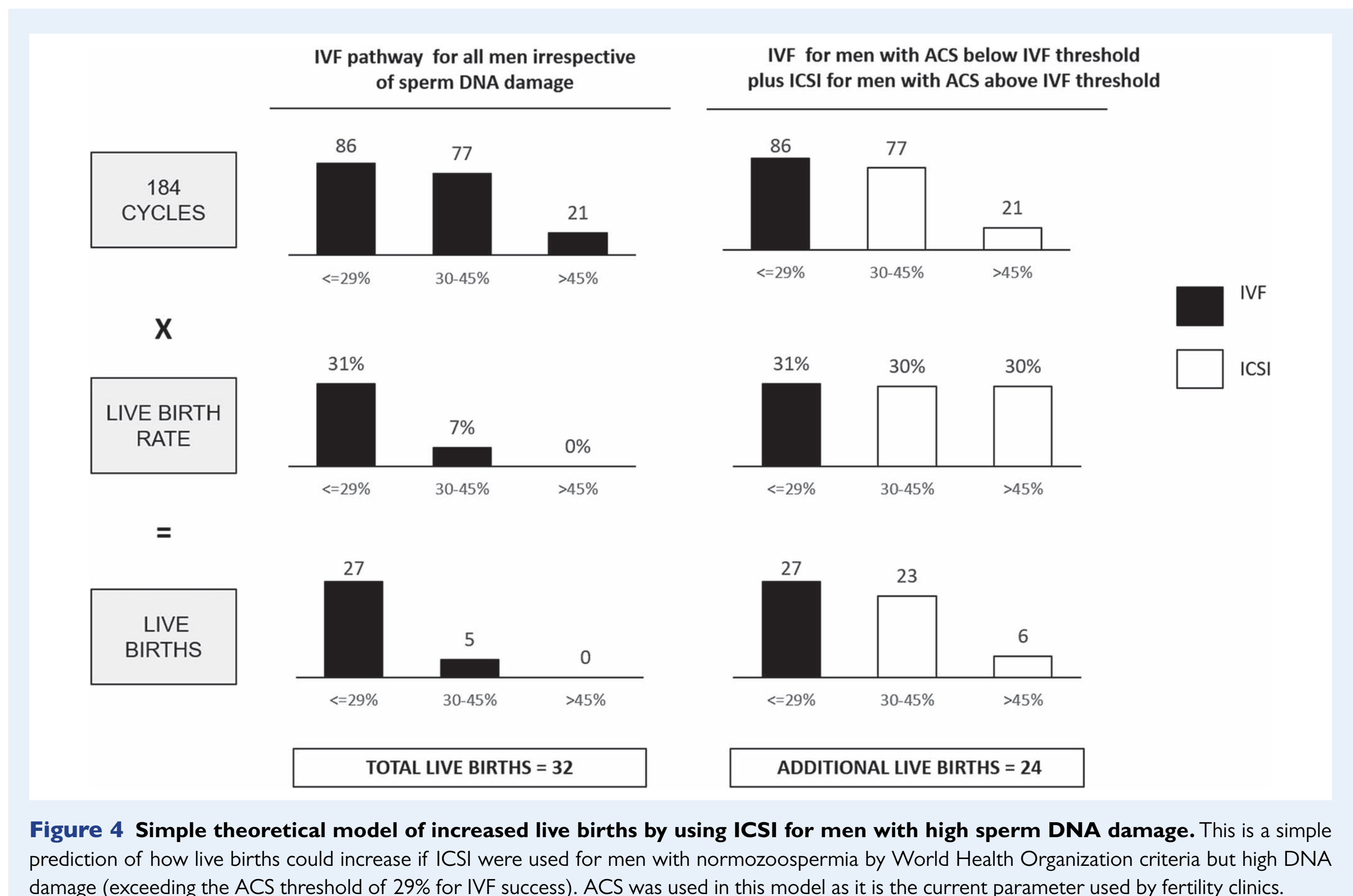

Figure 4 **Simple theoretical model of increased live births by using ICSI for men with high sperm DNA damage.** This is a simple prediction of how live births could increase if ICSI were used for men with normozoospermia by World Health Organization criteria but high DNA damage (exceeding the ACS threshold of 29% for IVF success). ACS was used in this model as it is the current parameter used by fertility clinics.

in this study and others (Lewis *et al.*, 2013; Meseguer *et al.*, 2011; Simon *et al.*, 2017) is its close association with ART outcomes (Simon *et al.*, 2014; Cissen *et al.*, 2016). Added to this is its requirement for only 5000 sperm per sample. It can also detect both double- and single-strand breaks in DNA that have previously been associated with both protamine- and histone-bound chromatin. The Comet assay has been recommended for its reproducibility, sensitivity and specificity (Simon *et al.*, 2014).

The novel parameters of HCS and LCS used here support previous studies (Simon *et al.*, 2017) using ACS that report sperm DNA fragmentation as a highly predictive biomarker of male infertility (Simon *et al.*, 2011). Given the high incidence of idiopathic infertility (~25%), this may be a useful tool in the fertility clinic for initial diagnosis and predicting ART outcomes. In couples who historically have been categorised as suffering from idiopathic male infertility, assessing DNA integrity may indicate underlying pathologies such as varicoceles, environmental factors or infections which, with treatment, may help to improve sperm DNA quality and outcomes in terms of natural conception and from ART. Currently, in many centres, these couples are directly offered IUI or IVF, with lower chances of success because of undiagnosed pathologies. This is neither cost effective nor in the best interests of the patient.

Both the sensitivity and specificity of the three Comet parameters were high (Table I) indicating their value as a diagnostic test for male infertility and particularly for infertile men with normal semen profiles, to identify hidden anomalies that reduce IVF success.

We were surprised by the homogeneity of sperm DNA quality in the population of fertile men, given the low values for normality in a semen analysis. All the parameters indicate that DNA fragmentation is a robust diagnostic measure of male infertility. The only property of sperm that matters post fertilization is genetic integrity, so a wide range of motility and morphology values may not be critical but DNA reliability still is.

Another focus of this study was to determine if there was an association between sperm DNA fragmentation and LBR following IVF. This data confirms, indirectly, a strong association with earlier fertility checkpoints such as fertilization and embryo quality as well as in implantation and live birth. Furthermore, in those cases where ACS fell around the threshold, the addition of the proportions of undamaged (LCS) and badly damaged sperm (HCS) improved the predictive value of the test. All the parameters were in line with each other, with HCS being the most significant (Table II IVF: $P = 0.003$; Table IV ICSI: $P = 0.018$).

From this study, it is clear that men with high levels of sperm DNA damage would be more likely to achieve a pregnancy with ICSI than IVF. With either treatment, if they have less than 15% damage (ACS), they have the highest chance of a live birth. However, if the sperm DNA damage (ACS) exceeds 27–29%, it would be advised to use ICSI, as live birth rates remain steady even though DNA damage increases, in contrast to IVF. This is in disagreement with several studies (Bhattacharya *et al.*, 2001; Li *et al.*, 2018) that conclude that ICSI does not increase the cumulative LBR in non-male factor

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infertility, suggesting that men with normal semen analyses should not be offered ICSI as it is no more successful than IVF for 'non-male factor' cases. However, data in this current study and others (Meseguer et al., 2011; Oleszczuk et al., 2013) show that a high proportion of men with normozoospermia have molecular anomalies in their sperm. Consequently, classifying male factor by semen analyses alone provides a misleading message and may lead to a less successful ART procedure.

There is wide consensus that female age is one of the most prognostic factors in ART success. With this is mind, female age and HCS were compared by logistic regression to see if the importance of female age over-rode the usefulness of male genomic testing. In IVF, the strength of HCS was such compared with female age that it would take 10 years of additional female age to negate the benefit of using the Comet HCS score. However, taking the two together is the best clinical approach. The non-significance of AMH adds to our hypothesis that it is not the woman's ovarian reserve that is critical but rather the poorer ability of older eggs to repair DNA damage. The data also shows that older eggs can be equally successful if used with good-quality sperm DNA. In ICSI, female age and sperm DNA quality both have significant, although less, impact on live births compared with IVF and should be included together in clinical decisions.

The differences seen in success rates between IVF and ICSI may be associated with the ability of the oocyte to repair sperm DNA damage before first cleavage. Unlike IVF, up to 30% of women undergoing ICSI have no underlying pathology. Thus, their oocytes may have more capacity to repair DNA damage even if the injected sperm is of poor quality. Meseguer et al. (2011) reported that high-quality oocytes from donors could offset the negative effect of sperm DNA damage on pregnancy. Further, in ICSI, the gametes are not subjected to prolonged culture so the sperm may have less damage than those exposed to culture media overnight, as in IVF procedures. Studies from Dumoulin et al. (2010) and Kleijkers et al. (2014) have shown that, for IVF babies, the birth weight and 2-year growth markers can be markedly influenced by minor differences in culture conditions. In contrast to IVF, ICSI sperm were injected into the optimal environment of the ooplasm within a few hours of ejaculation. This may protect both from laboratory-induced damage and allow the oocyte to begin repair earlier.

This is the first study to compare the accepted female age factor with an equivalent factor in the male. It is also the first study to use a DNA test to quantify subpopulations of sperm with varying amounts of DNA damage, within one semen sample. It is clear that this novel Comet parameter, namely HCS, indicating the proportion of sperm with a high level of damage has the greatest impact in reducing live births. This shows the importance of refining sperm DNA tests to use a test that detects the level of damage in individual sperm. Only the Comet assay can currently provide this information.

The study satisfies the challenge faced by any DNA test to identify clinical thresholds of fertile subpopulations with discriminatory power for infertility diagnosis and predictive power for ART. Given its usefulness, it should be added to all male workups as an adjunct to a semen analysis.

Study limitations

This was a retrospective study. In addition, an out-of-study prediction needs to be performed to assess the usefulness of COMET parameters. Given the low success rate in ART, sample sizes in such studies are always suboptimal. Little is known of male fertility history or confounding diseases or lifestyle factors.

Conclusion

The proportion of sperm with low or high levels of DNA damage, as assessed by the Comet assay, provides discriminatory information for male infertility diagnosis and prediction of both IVF and ICSI live births. This novel tool provides a pathway for personalised and optimised male fertility diagnosis and treatment in couples with unexplained and male infertility.

Authors' roles

SEML planned and designed the study. JN, KL and AVM were responsible for the data collection. KL conducted the main part of the analysis, while TY, SM, PL and JR contributed to the interpretation of the analysis. SEML and AVM drafted the article, while all authors critically revised the manuscript and approved the final version.

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Conflict of interest

Sheena Lewis and Kathryn Lee are employees of Examenlab Ltd, a university spin-out company with a commercial interest in sperm DNA damage. No other author has a conflict of interest to declare.

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