RESEARCH

Oxidative stress and DNA damage status in couples undergoing *in vitro* fertilization treatment

Iman Al-Saleh[1], Serdar Coskun[2], Reem Al-Rouqi[1], Tahreer Al-Rajudi[1], Chafica Eltabache[1], Mai Abduljabbar[1] and **Saad Al-Hassan[3]**

[1]Environmental Health Program, Research Centre, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia
[2]Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia
[3]Reproductive Medicine Unit, Department of Obstetrics and Gynecology, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

Correspondence should be addressed to I Al-Saleh: iman@kfshrc.edu.sa

Abstract

This study examined the status of oxidative stress in 599 couples undertaking *in vitro* fertilization (IVF) treatment and its association with reproductive hormones, smoking, and outcomes. Oxidative stress biomarkers such as malondialdehyde, 8-hydroxy-2-deoxyguanosine, hydrogen peroxide (H_2O_2), catalase (CAT), and total antioxidant capacity (TAC) were determined in follicular fluid and seminal plasma. Tail moment (TM) was used to evaluate DNA damage in the sperm and granulosa cells. Reproductive hormones in serum and cotinine (COT) in urine, follicular fluid, and seminal plasma samples were determined. Separate multivariate linear regression was used to assess associations between levels of each oxidative stress biomarker and each hormone and smoking parameter (modeled as natural log-transformed). The findings indicate that some oxidative stress and DNA damage biomarkers played a role in disrupting certain reproductive hormones in women and their male partners either by overproducing reactive oxygen species or reducing antioxidant defense capacity. Although women were nonsmokers, COT levels > 50 and 10 µg/L in urine and follicular were observed in 5.7 and 1.7%, respectively. Levels of follicular fluid COT were positively associated with H_2O_2 and TM. We used log-binomial multivariate regression to estimate relative risks for the association between oxidative stress/DNA damage and IVF binary outcomes (fertilization rate > 50%, biochemical pregnancy, clinical pregnancy, and live birth). An increase in the CAT levels of follicular fluid was associated with a 48 and 41% decrease in the risk of poor fertilization rate (≤50%) and unsuccessful live birth, respectively. After the models were adjusted for hormonal factors, the associations remained the same, except that the elevated TAC in follicular fluid became significantly associated with a decrease of 42% in the risk of poor fertilization rate (≤50%). The higher antioxidant activity (CAT and TAC) in follicular fluid might positively impact specific IVF outcomes.

Lay summary

Oxidative stress occurs when antioxidant molecules are insufficient in the body to destroy free radicals that can damage the cells, proteins and DNA, causing different health conditions, including infertility. The role of oxidative stress in female infertility has not received as much attention as male infertility, and research is still limited. This study explored whether the overproduction of free radicals can impact the success of in vitro fertilization (IVF) treatment using several biological markers such as hydrogen peroxide, catalase, and total antioxidant capacity. Our findings revealed that the high antioxidant levels in the fluid surrounding the egg were linked with a high fertilization rate. Additionally, oxidative stress status in couples was associated negatively with several reproductive hormones and smoking status. Biomarkers of oxidative stress and DNA damage might have potential applications in evaluating IVF patients' clinical characteristics such as causes of infertility, hormonal profile, fertilization rate, implantation and live birth.

Key Words: ▶ oxidative stress ▶ DNA damage ▶ follicular fluid ▶ seminal plasma ▶ smoking ▶ reproductive hormones ▶ IVF

Reproduction and Fertility (2021) **2** 117–139

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

Introduction

Oxidative stress is the result of an imbalance between reactive oxygen species (ROS) formation and enzymatic and non-enzymatic antioxidants, which may play a complex role in the pathogenesis of a range of human diseases, including neurodegenerative, cardiovascular, metabolic disorders, and cancer (Raghunath *et al.* 2018). The role of ROS in the etiology of male and female infertility has also been reported (Agarwal *et al.* 2012*a*, 2014). Some of the factors that have been implicated in ROS production are related to the modern lifestyle, for example, smoking, unhealthy eating habit, and lack of exercise, as these factors are associated with excess inflammation, oxidative stress, and ultimately DNA damage (Moller *et al.* 2014, da Silva 2016). Additionally, many environmental pollutants can induce ROS production, and thereby inducing a state of oxidative stress, which can impair testicular function (Asadi *et al.* 2017) and contribute to oocyte dysfunction (Devine *et al.* 2012) and also cause epigenetic modifications of gametes (Dattilo *et al.* 2016).

ROS such as superoxide anions, hydrogen peroxide (H_2O_2), and the hydroxyl free radical are formed as by-products of oxygen metabolism (Sharma *et al.* 2017). The presence of excess ROS can cause damage to lipids, proteins, and nucleotides (Freinbichler *et al.* 2011). Notably, ROS's overproduction has been found to significantly impact the success of *in vitro* fertilization (IVF) (Bedaiwy *et al.* 2012, Askoxylaki *et al.* 2013). A recent study revealed improved IVF outcomes in women under a moderate degree of oxidative stress (Rosen *et al.* 2019). Further, ROS measurement in seminal plasma has been suggested to have diagnostic and prognostic value in male infertility assessment (Venkatesh *et al.* 2011) since 30–80% of infertile men have elevated ROS levels (Agarwal & Allamaneni 2011). DNA is one of ROS's most common targets, which has been associated with cellular transformation and genome instability. However, 8-oxo-2′-deoxyguanosine (8-OHdG) is widely used as a biomarker for oxidative modifications of DNA bases (Lee & Pervaiz 2011). Additionally, 8-OHdG is considered as a specific, quantitative biomarker to determine the extent of the oxidative DNA damage caused by ROS in human sperm (Shen & Ong 2000), as oxidative damage is known to reduce spermatogenesis and sperm function, and eventually, lead to male infertility (Agarwal *et al.* 2014, Guerriero *et al.* 2014). Sperm DNA damage is a helpful biomarker for diagnosing male infertility and predicting assisted reproduction outcomes (Lewis *et al.* 2013, Simon *et al.* 2017). The alkaline comet assay has been proven to be a relatively simple and versatile tool for assessing DNA damage and determining the efficacy of DNA repair mechanisms (Gunasekarana *et al.* 2015). Oxidative stress might disturb the hypothalamus-pituitary-gonadal axis functions via its action on the reproductive hormones (Darbandi *et al.* 2018). For example, one study has shown that ROS generation induced by lead poisoning resulted in testosterone (T) suppression via impairment of the hypothalamus-pituitary-gonadal axis (Gandhi *et al.* 2017).

ROS role in female infertility has not received as much attention as in male infertility, and there is minimal research on the topic (Ruder *et al.* 2008). ROS might play an essential role in the initiation of apoptosis in antral follicles by depleting glutathione, which is usually required for male pronucleus formation at the time of fertilization and embryonic development to the blastocyst stage (Devine *et al.* 2012). Cumulus cells play an essential role in oocyte maturation, ovulation, and fertilization (Zhuo & Kimata 2001). Animal studies have found the cumulus–oocyte complex to be useful for testing the genotoxicity of environmental agents with the comet assay (Einaudi *et al.* 2014, Greco *et al.* 2015). However, these findings have not been confirmed in human studies.

Furthermore, oxidative stress may impact the production of steroid hormones, such as follicle-stimulating hormone (FSH) and estradiol (E_2), produced by granulosa cells, and subsequently lower the quality of oocytes and the success rate of IVF outcome (Seino *et al.* 2002, Avila *et al.* 2016).

In the present study, we measured several biomarkers of oxidative stress in follicular fluid and seminal plasma and DNA damage in sperm and granulosa cells in 599 couples undergoing IVF treatment to assess the potential relationships of these biomarkers with reproductive hormones, smoking status, and IVF outcomes.

Materials and methods

Study population

A total of 599 women and their male partners who were undergoing IVF treatment at King Faisal Specialist Hospital and Research Centre (KFSH&RC), Riyadh, Saudi Arabia were enrolled between 8 March 2015 and 15 January 2017 for treatment. The women's age range was 19–48 years old, and their male partners' age range was 25–73 years old. The main reasons for IVF treatment were female factors (16.4%), male factors (47.7%), combined male and female factors (14.7%), and preimplantation genetic diagnosis

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

(PGD) (14.4%). The cause of infertility was not evident in 6.8% of the cases. The couples signed an informed consent form at the recruitment time approved by the KFSH&RC Research Ethics Committee (RAC#2132024). Urine, follicular fluid, and seminal plasma samples were collected from each couple. Figure 1 depicts the data for the study population.

Sample collection

Spot urine samples

All the enrolled couples provided their urine samples during clinic visits either in the morning or afternoon in a sterile 100 mL polypropylene cup 2 to 3 days before follicle aspiration. The samples were aliquoted and stored at −20°C until the analysis of cotinine (COT).

Follicular fluid samples

Follicular aspirates without major blood contamination were obtained from each of the female participants. The follicular aspirate was poured into 60 mm Falcon dishes (Beckton Dickinson Labware, Franklin Lakes, New Jersey, USA), and cumulus–oocyte complexes were transferred to another dish containing Multipurpose Handling Medium (Irvine Scientific, Santa Ana, CA). The remaining granulosa cells and follicular fluids, which are usually discarded after this process, were separated and transferred to 1.5 mL vials and 15 mL conical tubes, respectively. The follicular fluid samples were centrifuged at 800 ***g*** for 5 min, and the supernatant was collected into 2 mL labeled polypropylene plastic tubes and stored at −20°C for malondialdehyde (MDA), 8-OHdG and ROS assays, as well as COT assay. The granulosa cells were transferred into 1.5 mL cryogenic vials (Corning® Incorporated, NY, USA) and centrifuged at 800 ***g*** for 5 min to separate the supernatant. The pellet was re-suspended in 150 µL of calcium- and magnesium-free PBS (Sigma Chemical Co.), and the vials were immediately placed in isopropanol progressive Nalgene® Mr Frosty freezing container (Sigma-Aldrich) at −80°C overnight, immersed in liquid nitrogen after 24 h, and used later for the comet assay.

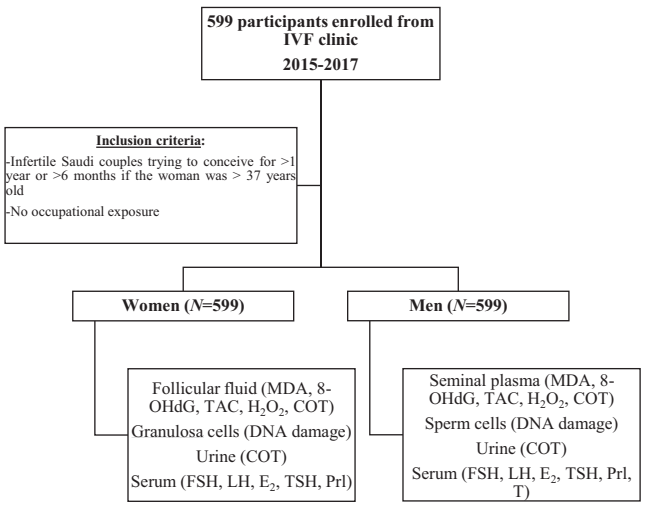

Figure 1 Flow chart of the study population.

Semen samples

On the same day, oocyte retrieval was performed, semen samples were collected by masturbation (following the semen collection instructions given to the patients) and allowed to liquefy for 15–30 min at room temperature. Before the sample preparation for IVF/intracytoplasmic sperm injection (ICSI), routine semen parameters were assessed. The remaining semen samples (250–500 µL), which are usually discarded, were placed in a 15 mL polypropylene tube within 30 min of collection. Seminal plasma was separated from spermatozoa by centrifugation at 800 ***g*** for 5 min and stored at −80°C for MDA, 8-OHdG, ROS and COT assays. The sperm pellets were transferred into 1.5 mL cryogenic vials and immediately placed in isopropanol progressive Nalgene® Mr Frosty freezing container at −80°C overnight and stored in liquid nitrogen the previous day before they were processed for the comet assay.

Analytical methods

Hormonal parameters

As part of the routine IVF procedure, a blood sample was drawn from each woman on the 3rd day of the menstrual cycle (follicular phase) for analysis of FSH, luteinizing hormone (LH), thyroid-stimulating hormone (TSH), E_2 and prolactin (Prl). A male hormonal profile, including LH, FSH, E_2, TSH, Prl, and T, was requested only in male infertility cases at the initial clinical visit. The data for these parameters were obtained from the patient medical records. In the case of normozoospermic men for whom the hormonal profile was not available in their medical records, a 4 mL venous blood sample was drawn on the day of oocyte retrieval into Vacutainer® tubes (Becton, Dickinson, and Co., NJ, USA) without anticoagulant and the serum fraction was analyzed at the Clinical Biochemistry, Pathology and Laboratory Medicine Department, KFSH&RC.

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

Oxidative stress and DNA damage biomarkers

Total antioxidant capacity (TAC), catalase (CAT) activity, and hydrogen peroxide (H_2O_2)/peroxidase were measured using the OxiSelect™ Total Antioxidant Capacity Assay Kit (Cell Biolabs, San Diego, USA), DetectX Catalase Fluorescence Activity Kit (Arbor Assays, MI, USA); and OxiSelect™ Hydrogen Peroxide/Peroxidase Assay Kit (Fluorometric), respectively. The 8-OHdG levels were measured in follicular fluid and seminal plasma using the HT 8-oxodG kit II (Trevigen Inc., Gaithersburg, USA). According to the manufacturer's instructions, all assays were performed, and absorbance was read using an automated ELISA microplate reader. The Anthos Zenyth 3100 Multimode Detector (Zenyth, Salzburg, Austria) was used for CAT and H_2O_2/peroxidase assay. TAC and 8-OHdG were measured using Biotek™ EL ×800™ Absorbance Microplate Readers (Winooski, USA). Finally, MDA assay was performed using the Alliance Waters HPLC 2695 system and a multi-fluorescence detector (Model 2475) as previously described (Al-Saleh *et al.* 2007).

Single-cell gel electrophoresis assay (comet assay)

The alkaline comet assay was performed in granulosa and sperm cells, according to the modified methods of Singh *et al.* (1988) and Lindley *et al.* (2001). Images were taken at a magnification of 20× with a fluorescence optical microscope (Nikon Eclipse TI-E; Nikon, Japan) equipped with excitation (465 nm) and barrier (595 nm) filters. Twenty-five cells per sample were randomly evaluated using the Comet assay IV Windows software with a monochrome CCD IEEE1394 FireWire video camera (Perceptive Instruments, Halstead, UK). Five parameters were measured: head length (HL), tail length (TL), head intensity (TI), percentage of DNA tail (% DNA tail), and tail moment (TM). The percentage of DNA in the tail multiplied by the distance between the center of the tail and head represents TM that was used in the present study as an indicator of DNA damage.

A limitation of the comet assay is the lack of standardized protocol that hinders the interpretation and comparison of the results with other studies (Collins 2015).

COT

The Cotinine ELISA immunoassay kit (Bio-Quant COTININE Direct ELISA, San Diego, USA) was used to measure COT in urine, follicular fluid, and seminal plasma as a biomarker of nicotine exposure. A 10 µL sample was aliquoted in duplicate into a 96-well microtiter plate and processed according to the manufacturer's protocol. Absorbance was measured at OD_{450} using a Biotek™ EL ×800™, Absorbance Microplate Reader (Winooski, USA).

Main study outcomes and potential confounders

In this study, we examined the association of oxidative stress and DNA damage markers with primary IVF outcomes such as fertilization rate (the percentage of the number of oocytes with 2PN divided by the total number of oocytes retrieved, and achieved fertilization rate was > 50%), biochemical pregnancy (positive β-human chorionic gonadotropin in urine and serum on day 14 with no evidence of gestational sac or fetal heartbeat to indicate continued pregnancy), clinical pregnancy (the presence of gestational sacs with fetal heartbeat confirmed by ultrasound) and live birth (successful delivery of one or more live neonates after 24 weeks of gestation). Each endpoint (fertilization rate, biochemical pregnancy, clinical pregnancy, and live birth) was dichotomized as a binary variable. A failure group represented women with a fertilization rate ≤ 50%, no biochemical pregnancy (negative β-human chorionic gonadotropin test), no clinical pregnancy (loss of gestational sac on ultrasound) or no live birth (unsuccessful delivery of a live neonate).

Of the 599 couples, 422 (70.5%), 196 (32.7%), 161 (26.9%) and 136 (22.7%) achieved fertilization (>50%), biochemical pregnancy, clinical pregnancy and live birth, respectively.

Potential confounders associated with IVF outcomes and/or oxidative stress, such as age, BMI, causes of infertility, pollution, socioeconomic status, and smoking, were selected based on previous reports (Benedict *et al.* 2011, Moller *et al.* 2014, Black *et al.* 2016, Kumar *et al.* 2018).

Statistical analysis

The data are presented as mean, median, and geometric mean. All parameters were naturally logarithm transformed (ln) to obtain a normal distribution for analysis. Pearson's correlation coefficients were calculated to determine the associations between the studied parameters. Separate multivariate linear regression models were established for each of the reproductive hormones and smoking parameters, which were used as continuous variables that were predicted by the ln-concentration of each of

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

the oxidative stress and DNA damage variables, with and without adjusting for confounders (age, BMI, causes of infertility, educational level, total monthly family income, regional distribution of residence and urinary COT levels). Results are expressed as the value of β standardized regression coefficients as estimates of effect, with 95% CI and *P* values used to assess statistical significance. We evaluated collinearity with the variance inflation factor (VIF) in the linear regression analyses. This did not exceed 10, the commonly used cut-off for collinearity problem, mainly when correlations between variables are not highly correlated (O'Brien 2007, Lavery *et al.* 2019). In our study, the correlations between variables were either small or moderate, and VIF values were less than 2.

Since the prevalence of achieving IVF endpoints (fertilization rate > 50%, biochemical pregnancy, clinical pregnancy, and live birth) was high (>10%) in our study, we used log-binomial multivariate regression models to estimate the relative risk (RR) and 95% CI as an alternative approach to logistic regression (Barros and Hirakata, 2003). We modeled the association between each binary IVF outcome and each oxidative stress and DNA damage parameter as a continuous variable (ln-transformed) after adjusting for potential confounders. Since hormonal abnormalities may contribute to infertility and unsuccessful IVF outcome (Vannuccini *et al.* 2016), we repeated the log-binomial regression analysis after including reproductive hormones as a potential confounder. Principal component analysis (PCA) was applied with varimax rotation to identify the main clusters of reproductive hormones in women and their male partners. The choice of principal components (PCs) was based on their eigenvalue: PCs with an eigenvalue greater than 1 were selected. The adequacy of PCA was verified using the Kaiser-Meyer-Olkin value, which should be higher than 0.5, and significance according to Bartlett's test. Factor loading ≥0.30 was set as the criterion for parameter retention. PCA yielded two components that explained 54.538% of the total variance in women's reproductive hormones with positive loadings for PC1 (FSH, LH, and E_2) and PC2 (TSH and Prl). In the case of the male participants, PCA identified three components that explained 69.413% of the total variance in reproductive hormones, with positive loadings for PC1 (FSH and LH and E_2) and PC2 (E_2 and T) but negative loadings for PC3 (TSH and Prl).

Significance was selected at $P < 0.05$, and all calculations were performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp.).

Results

Oxidative stress biomarkers, reproductive hormones, and smoking

The general characteristics of the 599 couples enrolled in this study are presented in Table 1. Biomarkers of oxidative stress (MDA, 8-OHdG, CAT, H_2O_2, and TAC) in seminal plasma and follicular fluid, DNA damage (HL, HI, TL, TI, and TM) in sperm and granulosa cells, and smoking (COT) in urine, follicular fluid, and seminal plasma are presented in Table 2. 8-OHdG, CAT, H_2O_2, and TAC were detected in all follicular fluid samples. In contrast, only CAT and H_2O_2 were detected in all seminal plasma samples. MDA was determined in 96.8 and 87.9% of follicular fluid and seminal plasma samples, respectively. 8-OHdG and TAC were found in 99.4 and 99.2% of seminal plasma samples, respectively.

%DNA tail, TM, and TL are the most frequently used comet parameters for assessing genotoxicity because of their ability to measure DNA damage (Hartmann & Speit 1997, Lee & Steinert 2003, Kumaravel *et al.* 2009). Strong correlations were found between TL and %DNA tail (r=0.778) and TM (r = 0.868) and between TM and %DNA tail (r = 0.985) ($P < 0.001$ for all) in granulosa cells from women. In sperm cells, strong correlations were detected between TL and %DNA tail (r=0.617) and TM (r = 0.772) and between TM and %DNA tail (r = 0.974) ($P < 0.001$ for all). In this study, TM was chosen for statistical evaluation as it provides the most stable estimates of DNA damage because of its uniformity in quantile dispersions (Lee *et al.* 2004).

COT levels were detected in more than 90% of urine samples from both women and their male partners. However, COT was detected in 49.3% (283) and 52.7% (77) of follicular fluid and seminal plasma samples, respectively. A 50 µg/L cut-off of COT level in urine was employed to distinguish active smokers from nonsmokers (Jarvis *et al.* 1987). Though the authors used gas chromatography for urinary COT analysis, a similar cut-off was determined with the ELISA method (Balhara *et al.* 2012). The highest COT levels were seen in urine, yet a similar magnitude was observed in other biological matrices such as blood and semen (Vine *et al.* 1993). In the present study, 349 (58.3%) men and 34 (5.7%) women had urinary COT level above 50 µg/L. There is no cut-off for COT in the seminal plasma or follicular fluid to the best of our knowledge. However, Fuentes *et al.* (2010) found that COT in recent smokers' follicular fluid was > 10 µg/L, whereas nonsmokers' levels were undetectable. Only ten (1.7%) of our women had

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

Table 1 Main socio-demographic, lifestyle and health characteristics among 599 IVF couples in Saudi enrolled between 2015 and 2017.

Variables	Women				Male partners				Couples, *n* (%)
	n (%)	Mean ± S.D.	Min-max	Reference values[a]	*n* (%)	Mean ± S.D.	Min-max	Reference values[a]	
Continuous									
Age (years)	599	32.76 ± 5.02	18.7–47.5		599	37.86 ± 7.39	25–72.5		
BMI (kg/m²)	599	28.56 ± 4.75	15.82–42.6		597	29.79 ± 6.1	15.43–50.78		
Duration trying to conceive (years)	599	6.19 ± 3.69	0.5–27						
FSH (IU/L)	592	6.22 ± 3.44	0.4–36.6	3.5–12.5	596	5.51 ± 5.87	0.1–40.6	1.5–12.4	
LH (IU/L)	589	5.57 ± 5.09	0.1–62.5	2.4–12.6	593	5.41 ± 3.61	0.1–40.8	1.7–8.6	
TSH (mU/L)	598	3.49 ± 12.39	0.01–243	0.27–4.2	591	2.93 ± 3.5_2	0.01–52.04	0.27–4.2	
E2 (pmole/L)	527	151.95 ± 533.11	1.29–9849.1	46–607	454	100.52 ± 64.21	0.86–782	28–156	
Prl (µg/L)	598	14.69 ± 19.32	0.14–412	4.1–18	593	45.22 ± 863.29	0.5–21032	4.1–18	
T (nmol/L)					562	13.73 ± 7	0.16–71.61		
Categorical									
Educational levels									
≤Intermediate	124 (20.7)				119 (19.9)				
Secondary and diploma	204 (34.1)				284 (47.4)				
≥University	271 (45)				196 (32.7)				
Work status									
Working	225 (37.6)				565 (94.3)				
Past or never	374 (62.4)				34 (5.7)				
Smoking cigarettes									
Yes	None				141 (23.5)				
No	None				458 (76.5)				
Smoking water pipes									
Yes	10 (1.7)				72 (12)				
No	589 (98.3)				527 (88)				
Living with a male partner and other family members who smo_{ke} cigarettes and water pipes									
Yes	219 (36.6)								
No	380 (63.4)								
Regional distribution of residence									
Central									161 (26.9)
Eastern									178 (29.7)
Western									77 (12.9)
Southern									120 (20.0)
Northern									63 (10.5)
Total monthly family income in Saudi riyals									
<5000									66 (11)
5001–10,000									193 (32.2)
>10,000									168 (28)
Irregular/unknown/refused									
Causes of infertility									
Female factor									98 (16.4)
Male factor									286 (47.7)
Combined									88 (14.7)
Unexplained factor									41 (6.8)
PGD									86 (14.4)

[a]Reference ranges used at KFSH&RC.

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

Table 2 Descriptive statistics of biomarkers in 599 couples.

	Women						Male partners					
Biomarkers	Matrix	*n*	Mean	Median	GM[a]	Max	Matrix	*n*	Mean	Median	GM[a]	Max
8-OHdG (µg/L)	FF	562	22.401	14.784	17.244	143.800	SP	501	175.585	138.445	142.533[c]	960.317
MDA (nmol/mL)	FF	594	0.864	0.837	0.814[b]	5.138	SP	580	1.021	1.010	1.091[c]	8.011
CAT (U/mL)	FF	569	14.816	12.060	13.144	165.107	SP	224	11.160	9.206	10.132	56.813
H_2O_2 (µg/L)	FF	574	6.065	5.792	5.931	18.078	SP	232	27.532	24.618	25.161	97.726
TAC (µM)	FF	574	728.138	741.962	676.610	1594.687	SP	251	587.382	543.748	504.503[c]	2061.600
TL	FF	481	73.339	70.64	70.246	148.60	SP	278	59.217	58.5	56.596[c]	115.04
% DNA tail	GC	481	20.122	14.293	15.107	83.679	SC	278	16.263	15.095	14.002[c]	46.965
TM	GC	481	8.599	4.967	5.544	57.199	SC	278	5.248	4.483	4.193[c]	21.766
COT (µg/L)	FF	574	2.442	0.000	0.278[b]	271.298	SP	146	63.002	0.603	46.408[c]	316.897
COT (µg/L)	Urine	599	46.946	3.300	3.793[b]	8138.636	Urine	599	2764.453	91.948	175.097[c]	57894.391

[a]Geometric mean calculated after deleting zero values for MDA ($n = 19$), urinary COT ($n = 53$), COT ($n = 291$) in women[b] and MDA ($n = 70$), TL ($n = 1$), TI ($n = 1$), TM ($n = 1$), 8-OHdG ($n = 3$), TAC ($n = 2$), urinary COT ($n = 23$) and COT ($n = 69$) in male partners[c].
FF, follicular fluid; GC, granulosa cells; GM, geometric mean; SC, sperm cells; SP, seminal plasma.

COT in follicular fluid above 10 µg/L in the range between 19.37 and 271.3 µg/L. Terzioğlu *et al.* (2016) found COT levels in seminal plasma of nonsmokers and passive smokers were <10 µg/L. In our study, 61 (41.8%) men had COT higher than 10 µg/L in the range between 11.7 and 316.9 µg/L. Both Fuentes *et al.* (2010) and Terzioğlu *et al.* (2016) measured COT using the ELISA method. ELISA is considered a practical alternative screening assay to chromatographic methods. Kuo *et al.* (2002) found that the levels of urinary COT measured by HPLC were strongly correlated ($r > 0.9$) with levels measured using ELISA or gas chromatography.

Statistically significant inter-correlations between the tested biomarkers are shown in Table 3. The data indicate the presence of oxidative stress-related biomarkers mechanisms in the participating couples.

Associations of biomarkers of oxidative stress/DNA damage with reproductive hormones and smoking

We first examined the crude relationship between oxidative stress and DNA damage biomarkers and reproductive hormones in women and their male partners (Table 4). The levels of LH in women was positively associated with the 8-OHdG ($P = 0.014$) and CAT ($P = 0.001$) levels in follicular fluid, but it was negatively associated with TM in granulosa cells ($P < 0.001$). The TSH and E_2 levels were inversely related to follicular fluid H_2O_2 levels ($P = 0.007$) and TM in granulosa cells ($P = 0.029$), respectively. TAC in follicular fluid was inversely associated with Prl ($P = 0.045$). In women's urine, COT was positively correlated with follicular CAT levels ($P = 0.045$). Additionally, the follicular fluid COT level was positively correlated with the levels of follicular fluid H_2O_2 ($P = 0.05$) and TM in granulosa cells ($P = 0.012$). In the male partners, the FSH and LH levels were inversely associated with seminal MDA levels ($P < 0.001$ and $P < 0.039$, respectively). While the E_2 levels were positively correlated with seminal CAT levels ($P = 0.047$), T was inversely correlated with seminal TAC levels ($P = 0.024$). In the male partners' urine samples, the COT levels were positively and inversely correlated with CAT's seminal plasma levels ($P = 0.035$) and H_2O_2 ($P = 0.007$). None of the oxidative stress and DNA damage parameters were significantly correlated with COT in seminal plasma.

We adjusted all the regression models in Table 4 for age, BMI, ln-COT in urine, educational level, total family income, and cause of infertility. In women, LH remained significantly and positively associated with 8-OHdG ($P = 0.011$) and CAT ($P = 0.003$) in follicular fluid, but

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

Table 3 Inter-correlations among log-transformed oxidative stress biomarkers in women and their male partners (the values in bold denote significant correlations).

	MDA	8-OHdG	CAT	H_2O_2	TAC	TM
Women						
8-OHdG (μg/L)						
r	−0.074					
P	0.082					
n	562					
CAT (U/mL)						
r	−0.018	**0.114****				
P	0.671	**0.007**				
n	569	**556**				
H_2O_2 (μg/L)						
r	**−0.140****	**−0.216****	**0.131****			
p	**0.001**	**<0.001**	**0.002**			
n	**574**	**560**	**568**			
TAC (μM)						
r	**0.177****	-0.043	**0.101***	0.041		
P	**<0.001**	0.312	**0.016**	0.332		
n	**574**	560	**568**	573		
TM						
r	**0.147****	**−0.142****	−0.038	0.038	**0.111***	
P	**0.001**	**0.002**	0.410	0.403	**0.015**	
n	**481**	**468**	476	480	**480**	
Male partners						
8-OHdG (μg/L)						
r	**0.179****					
P	**<0.001**					
n	**498**					
CAT (U/mL)						
r	0.127	**0.209****				
P	0.058	**0.002**				
n	224	**222**				
H_2O_2 (μg/L)						
r	**0.306****	**0.194****	0.107			
P	**<0.001**	**0.003**	0.171			
n	**232**	**227**	164			
TAC (μM)						
r	0.088	0.083	0.031	**0.250****		
P	0.167	0.192	0.718	**0.002**		
n	249	246	139	**150**		
TM						
r	0.076	**0.213****	−0.061	0.194*	−0.069	
P	0.209	**<0.001**	0.504	0.022	0.407	
n	276	**271**	124	139	148	

*Correlation is significant at the 0.05 level (two-tailed); **Correlation is significant at the 0.01 level (two-tailed).

inversely correlated with TM in granulosa cells ($P < 0.001$). Further, TSH maintained an inverse correlation with H_2O_2 in follicular fluid ($P = 0.004$), but it became significantly and positively associated with 8-OHdG ($P = 0.022$). E_2 in follicular fluid remained negatively associated with TM in granulosa cells ($P = 0.032$). In contrast, Prl and TAC's relationship in follicular fluid became insignificant ($P = 0.071$) after adjusting the model for confounders. In the male partners, FSH remained negatively associated with MDA ($P < 0.001$). On the other hand, LH and MDA's relationship and between E2 and CAT disappeared after adjusting for confounders. We also observed positive and significant correlations between T and MDA ($P = 0.026$) that were not observed in the crude regression analysis. About the relationship between biomarkers of oxidative stress and DNA damage and COT in urine, follicular fluid, and seminal plasma after adjusting for confounders, the associations between follicular fluid CAT and urinary COT in women remained significant ($P = 0.022$), as did the correlation between follicular COT and H_2O_2 ($P = 0.047$)

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

Table 4 Crude and adjusted multiple linear regression modelstesting associations of biomarkers of oxidative stress and DNA damage and reproductive hormones in women and their male partners. Values represent β, regression coefficients, 95% CI, and *P*, the level of significance. β value corresponds to 1-ln unit change in oxidative stress and DNA damage. The values in bold denote significant associations.

	MDA (nmol/mL)	**8-OHdG** (μg/L)	**CAT** (U/mL)	**H_2O_2** (μg/L)	**TAC** (μM)	**TM**
Women						
Reproductive hormones						
FSH	−0.007 (−0.093, 0.078), 0.862	−0.005 (−0.111, 0.098), 0.905	0.031 (−0.043, 0.094), 0.464	−0.014 (−0.037, 0.026), 0.741	−0.032 (−0.093, 0.041), 0.446	−0.015 (−0.185, 0.133), 0.748
Adjusted[a]	−0.021 (-0.111, 0.066), 0.616	0.026 (−0.073, 0.138), 0.545	0.037 (−0.041, 0.102), 0.407	−0.016 (−0.039, 0.027), 0.707	−0.042 (−0.103, 0.035), 0.335	−0.04 (−0.236, 0.094), 0.397
LH	−0.063 (−0.092, 0.012), 0.127	**0.105 (0.016, 0.141), 0.014**	**0.137 (0.027, 0.11), 0.001**	0.044 (−0.009, 0.03), 0.297	−0.07 (−0.075, 0.006), 0.095	**−0.184 (−0.293, −0.102), <0.001**
Adjusted[a]	−0.064 (−0.094, 0.012), 0.133	**0.109 (0.019, 0.144), 0.011**	**0.131 (0.023, 0.108), 0.003**	0.03 (−0.013, 0.027), 0.407	−0.063 (−0.073, 0.011), 0.144	**−0.206 (−0.321, −0.122), <0.001**
TSH	0.032 (−0.035, 0.08), 0.437	0.071 (−0.01, 0.129), 0.093	−0.02 (−0.057, 0.035), 0.641	**−0.112 (−0.05, −0.008), 0.007**	0.002 (−0.043, 0.046), 0.961	0.023 (−0.091, 0.154), 0.614
Adjusted[a]	0.03 (−0.037, 0.079), 0.473	**0.095 (0.012, 0.148), 0.022**	−0.008 (−0.051, 0.042), 0.852	**−0.121 (−0.052, −0.01), 0.004**	0.004 (−0.043, 0.047), 0.924	0.021 (−0.11, 0.137), 0.832
E_2	0.001 (−0.043, 0.044), 0.985	0.05 (−0.023, 0.084), 0.262	0.067 (−0.008, 0.059), 0.133	0.013 (−0.014, 0.019), 0.777	−0.042 (−0.05, 0.017), 0.345	**−0.106 (−0.168, −0.009), 0.029**
Adjusted[a]	0.005 (−0.042, 0.047), 0.909	0.048 (−0.025, 0.083), 0.288	0.088 (−0.001, 0.068), 0.058	0.003 (−0.016, 0.017), 0.951	−0.051 (−0.054, 0.015), 0.263	**−0.108 (−0.173, −0.008), 0.032**
Prl	−0.044 (−0.089, 0.026), 0.282	0.047 (−0.03, 0.109), 0.262	0.014 (−0.038, 0.053), 0.734	−0.01 (−0.024, 0.019), 0.814	**−0.084 (−0.089, −0.001), 0.045**	0.069 (−0.024, 0.188), 0.131
Adjusted[a]	−0.039 (−0.086, 0.03), 0.343	0.064 (−0.015, 0.121), 0.128	−0.004 (−0.049, 0.044), 0.925	−0.015 (−0.025, 0.017), 0.729	−0.076 (−0.086, 0.004), 0.071	0.069 (−0.027, 0.191), 0.138
Smoking parameters						
Urinary COT	−0.048 (−0.041, 0.01), 0.241	0.053 (−0.011, 0.051), 0.207	**0.084 (0, 0.041), 0.045**	0.039 (−0.005, 0.014), 0.356	0.029 (−0.013, 0.027), 0.489	0.019 (−0.037, 0.056), 0.678
Adjusted[a]	−0.05 (−0.043, 0.01), 0.237	0.051 (−0.013, 0.051), 0.235	**0.101 (0.004, 0.046), 0.022**	0.044 (−0.005, 0.015), 0.306	0.025 (−0.015, 0.027), 0.57	0.007 (−0.044, 0.052), 0.879
Follicular fluid COT	−0.026 (−0.032, 0.021), 0.669	−0.044 (−0.075, 0.034), 0.46	0.1 (−0.004, 0.053), 0.094	**0.117 (0, 0.028), 0.05**	0.057 (−0.016, 0.045), 0.338	**0.163 (0.021, 0.163), 0.012**
Adjusted[a]	−0.035 (−0.035, 0.019), 0.574	−0.021 (−0.063, 0.043), 0.714	0.088 (−0.008, 0.051), 0.153	**0.118 (0, 0.028), 0.047**	0.049 (−0.018, 0.044), 0.412	**0.164 (0.02, 0.165), 0.012**
Men						
Reproductive hormones						
FSH	**−0.297 (−0.296, −0.161), <0.001**	−0.051 (−0.125, 0.033), 0.254	−0.006 (−0.078, 0.071), 0.911	−0.07 (−0.113, 0.034), 0.292	−0.078 (−0.212, 0.049), 0.219	−0.094 (−0.235, 0.027), 0.118
Adjusted[a]	**−0.213 (−0.255, −0.11), <0.001**	−0.014 (−0.099, 0.072), 0.764	−0.008 (−0.088, 0.079), 0.936	−0.084 (−0.128, −0.033), 0.244	−0.08 (−0.232, 0.068), 0.267	−0.075 (−0.221, 0.054), 0.234
LH	**−0.086 (−0.169, −0.004), 0.039**	−0.03 (−0.13, 0.065), 0.511	0.015 (−0.087, 0.109), 0.825	−0.028 (−0.107, 0.07), 0.677	−0.038 (−0.209, 0.112), 0.553	−0.063 (−0.26, 0.08), 0.299
Adjusted[a]	−0.058 (−0.14, 0.024), 0.166	−0.009 (−0.109, 0.089), 0.845	0.02 (−0.087, 0.116), 0.799	−0.029 (−0.111, 0.071), 0.67	−0.033 (−0.207, 0.122), 0.61	−0.026 (−0.213, 0.139), 0.678
Male partners						
Reproductive hormones						
TSH	0.068 (−0.013, 0.135), 0.106	−0.032 (−0.116, 0.054), 0.473	−0.032 (−0.107, 0.065), 0.633	−0.011 (−0.082, 0.069), 0.867	0.098 (−0.033, 0.279), 0.122	−0.097 (−0.21, 0.021), 0.107
Adjusted[a]	0.047 (−0.031, 0.116), 0.255	−0.391 (−0.124, 0.05), 0.402	−0.015 (−0.098, 0.079), 0.832	−0.052 (−0.109, 0.048), 0.451	0.049 (−0.104, 0.225), 0.467	−0.062 (−0.182, 0.062), 0.331
E_2	−0.083 (−0.179, 0.011), 0.082	−0.019 (−0.124, 0.085), 0.712	**0.148 (0.001, 0.187), 0.047**	−0.035 (−0.11, 0.068), 0.64	−0.093 (−0.224, 0.047), 0.2	−0.032 (−0.183, 0.112), 0.636
Adjusted[a]	−0.09 (−0.19, 0.007), 0.069	−0.022 (−0.133, 0.087), 0.681	0.091 (−0.041, 0.158), 0.248	−0.033 (−0.115, 0.075), 0.675	−0.122 (−0.255, 0.023), 0.1	−0.029 (−0.189, 0.125), 0.69
Prl	−0.023 (−0.124, 0.071), 0.59	0.037 (−0.057, 0.14), 0.408	−0.028 (−0.135, 0.089), 0.684	0.028 (−0.091, 0.14), 0.676	0.072 (−0.08, 0.292), 0.262	0.062 (−0.064, 0.202), 0.308
Adjusted[a]	−0.029 (−0.132, 0.062), 0.478	0.028 (−0.069, 0.132), 0.563	−0.011 (−0.124, 0.105), 0.871	−0.003 (−0.123, 0.117), 0.96	0.064 (−0.097, 0.287), 0.331	0.061 (−0.067, 0.204), 0.322
T	0.069 (−0.02, 0.203), 0.108	0.084 (−0.009, 0.249), 0.069	0.093 (−0.038, 0.205), 0.177	−0.123 (−0.238, 0.011), 0.073	**−0.15 (−0.419, −0.03), 0.024**	−0.042 (−0.275, 0.134), 0.497
	0.089 (0.007, 0.231), 0.038	**0.109 (0.019, 0.293), 0.026**	0.09 (−0.053, 0.216), 0.232	−0.112 (−0.239, 0.032), 0.133	−0.111 (−0.39, 0.055), 0.139	−0.036 (−0.278, 0.159), 0.59
Smoking parameters						
Urinary COT	−0.065 (−0.03, 0.003), 0.116	−0.079 (−0.033, 0.002), 0.078	**0.141 (0.001, 0.034), 0.035**	**−0.176 (−0.04, −0.006), 0.007**	−0.112 (−0.051, 0.003), 0.078	0.036 (−0.018, 0.033), 0.556
Adjusted[a]	−0.041 (−0.025, 0.008), 0.327	−0.075 (−0.033, 0.003), 0.104	0.141 (−0.001, 0.036), 0.057	**−0.15 (−0.038, −0.002), 0.033**	−0.07 (−0.05, 0.008), 0.15	0.046 (−0.016, 0.035), 0.449
Seminal plasma COT	0.105 (−0.025, 0.069), 0.918	0.126 (−0.031, 0.107), 0.275	0.097 (−0.042, 0.092), 0.458	0.187 (−0.018, 0.095), 0.18	−0.034 (−0.156, 0.121), 0.801	0.263 (−0.014, 0.206), 0.085
Adjusted[a]	0.147 (−0.027, 0.087), 0.292	0.248 (−0.002, 0.153), 0.057	0.157 (−0.045, 0.126), 0.348	**0.473 (0.026, 0.168), 0.009**	−0.082 (−0.225, 0.142), 0.649	0.229 (−0.065, 0.233), 0.257

[a]*Each model was adjusted for* BMI (kg/m²), age (years) educational level (≤intermediate/secondary and diploma/university and above), total monthly family income (<5000/5000–10,000 (reference group)/>10,000/refused or unknown or irregular), regional distribution of residence (central/eastern (reference group)/western/southern/northern], causes of infertility (female factor/male factor (reference group/combined/unexplained factor/PGD), and ln-COT (μg/L).

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

and TM in granulosa cells ($P=0.012$). In the male partners, seminal plasma H_2O_2 remained significantly associated with urinary COT ($P=0.033$) and seminal COT ($P=0.009$), although the latter correlation was not significant in the crude analysis.

Association between oxidative stress and IVF outcomes

As shown in Table 5, multivariate log-binomial regression analyses revealed that there was a significant decrease in the risk of poor fertilization rate (≤50%) (RR=0.52, 95% CI=0.331, 0.817, $P=0.005$) and unsuccessful live birth (RR=0.592, 95% CI=0.377, 0.93, $P=0.023$) in association with one-unit increase in the follicular fluid CAT level. After adjusting the model for hormonal factors, as represented by PC1 (FSH, LH, and E_2), a decrease in the risk of poor fertilization rate (≤50%) was observed as a one-unit increase in the follicular fluid levels of CAT (RR=0.508, 95% CI=0.306, 0.842, $P=0.009$) and TAC (RR=0.585, 95% CI=0.493, 1.13, $P=0.023$), while the correlation between a decrease in the risk of unsuccessful live birth rate and per unit increase in the level of CAT in follicular fluid became insignificant (RR=0.623, 95% CI=0.367, 1.056, $P=0.079$). When the model was adjusted for PC2 (TSH and Prl), the risk of poor fertilization rate decreased in association with elevated follicular fluid levels of CAT (RR=0.479, 95% CI=0.289, 0.792, $P=0.004$) and TAC (RR=0.605, 95% CI=0.381, 0.961, $P=0.033$). In contrast, the relationship between CAT levels in follicular fluid and low risk of unsuccessful live birth became significant (RR=0.59, 95% CI=0.349, 0.995), $P=0.048$). In the male partners, none of the associations were significant with and without adjusting for PC1 (FSH and LH), PC2 (E_2 and T), or PC3 (TSH, Prl, and T). In Table 5, we only report the results for models adjusted with and without PC1, which showed the highest variability of 33.052% in women and 28.51% in their male partners.

Discussion

Oxidative stress profile in couples

Several researchers have used MDA levels in the follicular fluid as an index of lipid peroxidation and found it to be associated with polycystic ovary syndrome (Fatemi *et al.* 2017), endometriosis (de Lima *et al.* 2017), low response ovarian stimulation (Nunez-Calonge *et al.* 2016), anovulation (Kazemi *et al.* 2014), tubal infertility (Singh *et al.* 2013) and embryo quality (Liu *et al.* 2021). Our mean MDA value in follicular fluid (0.864 nmol/mL) was slightly higher than those reported by Artimani *et al.* (2018) in women with polycystic ovary syndrome (0.77 nmol/mL) but lower than Uppangala *et al.* (2020)'s value of 3.76 nmol/mL in women with a poor ovarian response, Thaker *et al.* (2020)'s value of 1.76 nmol/mL in women with positive IVF outcome, and Kazemi (2015)'s value of 0.98 nmol/mL in anovulatory women. However, our MDA value represents all women that underwent IVF treatment, of which only 186 (31.3%) of them had infertility problem (including those with combined male and female infertility factors). The MDA levels in the follicular fluid of women with infertility problems were 0.895 nmol/mL, which was not significantly different from other women who have undergone IVF treatment (0.850 nmol/mL), $P=0.277$. The same was seen after excluding cases with combined infertility factors. Jozwik (1999) observed low lipid peroxidation in the follicular fluid that might be related to the presence of efficient antioxidant defense systems surrounding the oocyte before ovulation. However, since MDA was detected in 96.8% of the follicular fluid samples, it could be deduced that some level of oxidative stress in the follicles might have impacted the IVF outcomes.

The MDA in seminal plasma can reflect the degree of peroxidative damage to spermatozoa (Agarwal *et al.* 2014). In general, our MDA value (1.02 nmol/mL) was slightly higher than the value of 0.94 nmol/mL (infertile) reported by Colagar *et al.* (2009) but lower than many other studies (Collodel *et al.* 2015, Atig *et al.* 2017, Josarayi *et al.* 2017). Our seminal plasma MDA values represent all men who have undergone IVF treatment. The seminal plasma MDA levels in 358 (61.7%) men with infertility problems (1.0 nmol/mL) (including those with combined male and female infertility factors) were significantly lower than others (1.102 nmol/mL) ($P< 0.001$). The same results were seen after excluding cases with combined infertility; seminal plasma MDA levels were significantly lower in men with male infertility (0.976 nmol/mL) vs others (1.098 nmol/mL) with $P < 0.001$. Similar results were reported by Layali *et al.* (2015), though most of the literature indicated higher seminal plasma MDA in infertile men (Agarwal *et al.* 2016). MDA in semen can be influenced by smoking (Chari & Colagar 2011), and 23.5% of men in the present study were smokers. After the smokers were excluded, the results remained the same: infertile men had significantly higher MDA levels than normozoospermic men. Additionally, sufficient antioxidant levels might lower the seminal plasma MDA

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

Table 5 Adjusted RRs and 95% CIs for %fertilization rate, biochemical pregnancy, clinical pregnancy, and live birth in association with biomarkers using binomial regression estimates (each modeled separately). The values in bold denote significant associations.

Biomarkers	%Fertilization rate	Biochemical pregnancy	Clinical pregnancy	Live birth
MDA-women				
Adjusted for factors[a] + ln-COT*	0.885 (0.645, 1.22), 0.45	1.161 (0.843, 1.60), 0.36	1.139 (0.813, 1.60), 0.45	1.139 (0.797, 1.63), 0.47
Adjusted for factors[a] + ln-COT* and PC1[b]	0.914 (0.647, 1.291), 0.611	1.167 (0.825, 1.652), 0.383	1.198 (0.832, 1.727), 0.331	1.171 (0.791, 1.735), 0.43
MDA-male partners				
Adjusted for factors[a] + ln-COT*	0.866 (0.665, 1.129), 0.288	0.974 (0.739, 1.283), 0.851	1.091 (0.818, 1.457), 0.552	1.145 (0.847, 1.548), 0.38
Adjusted for factors[a] + ln-COT* and PC1[c]	0.968 (0.693, 1.354), 0.851	0.95 (0.677, 1.334), 0.767	1.063 (0.741, 1.525), 0.74	1.052 (0.721, 1.535), 0.792
8-OHdG-women				
Adjusted for factors[a] + ln-COT*	0.822 (0.613, 1.10), 0.189	0.805 (0.603, 1.08), 0.142	0.784 (0.577, 1.07), 0.12	0.778 (0.563, 1.08), 0.128
Adjusted for factors[a] + ln-COT* and PC1[b]	0.773 (0.559, 1.07), 0.121	0.855 (0.625, 1.171), 0.33	0.825 (0.591, 1.151), 0.258	0.803 (0.564, 1.145), 0.226
8-OHdG-male partners				
Adjusted for factors[a] + ln-COT*	0.927 (0.683, 1.257), 0.625	0.734 (0.536, 1.005), 0.054	0.721 (0.516, 1.007), 0.055	0.746 (0.523, 1.064), 0.105
Adjusted for factors[a] + ln-COT* and PC1[c]	0.929 (0.647, 1.334), 0.691	0.706 (0.488, 1.021), 0.064	0.721 (0.487, 1.066), 0.101	0.756 (0.5, 1.145), 0.186
CAT-women				
Adjusted for factors[a] + ln-COT*	**0.52 (0.331, 0.817), 0.005**	0.853 (0.563, 1.29), 0.454	0.72 (0.466, 1.11), 0.137	**0.592 (0.377, 0.93), 0.023**
Adjusted for factors[a] + ln-COT* and PC1[b]	**0.508 (0.306, 0.842), 0.009**	0.857 (0.533, 1.377), 0.524	0.755 (0.459, 1.243), 0.269	**0.623 (0.367, 1.056), 0.079**
CAT-male partners				
Adjusted for factors[a] + ln-COT*	0.784 (0.362, 1.695), 0.536	0.589 (0.254, 1.368), 0.218	0.588 (0.252, 1.369), 0.218	0.753 (0.308, 1.841), 0.534
Adjusted for factors[a] + ln-COT* and PC1[c]	0.661 (0.236, 1.849), 0.43	0.772 (0.26, 2.294), 0.641	0.765 (0.248, 2.353), 0.64	0.828 (0.273, 2.513), 0.739
H_2O_2-women				
Adjusted for factors[a] + ln-COT*	0.917 (0.369, 2.28), 0.852	0.674 (0.274, 1.66), 0.39	0.594 (0.231, 1.53), 0.522	0.487 (0.18, 1.32), 0.156
Adjusted for factors[a] + ln-COT* and PC1[b]	0.806 (0.302, 2.152), 0.666	0.913 (0.348, 2.399), 0.854	0.714 (0.258, 1.974), 0.516	0.582 (0.198, 1.716), 0.327
H_2O_2-male partners				
Adjusted for factors[a] + ln-COT*	1.096 (0.534, 2.249), 0.802	0.888 (0.417, 1.891), 0.757	0.786 (0.36, 1.716), 0.545	0.744 (0.319, 1.735), 0.494
Adjusted for factors[a] + ln-COT* and PC1[c]	1.651 (0.707, 3.854), 0.246	1.088 (0.469, 2.526), 0.844	0.914 (0.383, 2.178), 0.838	0.936 (0.372, 2.353), 0.888
TAC-women				
Adjusted for factors[a] + ln-COT*	0.748 (0.493, 1.13), 0.171	0.853 (0.55, 1.32), 0.478	0.938 (0.594, 1.48), 0.784	1.049 (0.653, 1.68), 0.844
Adjusted for factors[a] + ln-COT* and PC1[b]	**0.585 (0.368, 0.93), 0.023**	0.745 (0.451, 1.23), 0.25	0.731 (0.428, 1.248), 0.251	0.829 (0.477, 1.441), 0.507
TAC-male-partners				
Adjusted for factors[a] + ln-COT*	1.054 (0.664, 1.673), 0.823	1.052 (0.688, 1.608), 0.815	1.047 (0.684, 1.602), 0.834	0.917 (0.548, 1.537), 0.743
Adjusted for factors[a] + ln-COT* and PC1[c]	1.872 (0.854, 4.102), 0.117	1.413 (0.679, 2.94), 0.356	1.125 (0.542, 2.332), 0.753	0.938 (0.431, 2.039), 0.871
TM-women				
Adjusted for factors[a] + ln-COT*	1.087 (0.875, 1.35), 0.452	0.958 (0.77, 1.19), 0.698	0.924 (0.734, 1.16), 0.5	1.062 (0.829, 1.36), 0.635
Adjusted for factors[a] + ln-COT* and PC1[b]	1.017 (0.8, 1.293), 0.889	0.951 (0.75, 1.206), 0.68	0.92 (0.717, 1.181), 0.514	1.03 (0.785, 1.351), 0.83
TM-male partners				
Adjusted for factors[a] + ln-COT*	1.073 (0.722, 1.595), 0.727	0.775 (0.509, 1.18), 0.235	**0.63 (0.391, 1.013), 0.057**	0.678 (0.406, 1.132), 0.137
Adjusted for factors[a] + ln-COT* and PC1[c]	1.065 (0.641, 1.771), 0.805	0.767 (0.459, 1.281), 0.31	0.584 (0327, 1.042), 0.069	0.708 (0.387, 1.293), 0.261

[a]Age (years), BMI (kg/m^2), causes of infertility (Female factor/male factor (reference group)/combined/unexplained factor/PGD), regional distribution of residence (central/eastern (reference group)/western/southern/northern), educational level (≤intermediate/secondary and diploma/university and above (reference group)), total monthly family income (<5000/5000–10,000 (reference group)/>10,000/refused or unknown or irregular); [b]PC1 (FSH, LH and E2); [c]PC1 (FSH and LH). *ln-COT (µg/L).

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

levels in infertile men (Agarwal & Sekhon 2011). One should mention that though MDA has been widely used as an indicator of oxidative stress, its specificity and sensitivity are still under debate (Grotto *et al.* 2009).

Excessive exposure to oxidative stress can result in oxidative DNA modifications, and one of the by-products is 8-OHdG, which is a popular marker of oxidative stress and oxidative-induced DNA damage (Dabrowska & Wiczkowski 2017). Several studies have measured 8-OHdG levels in blood and urine to understand the role of oxidative DNA damage in disease pathogenesis (Evans *et al.* 2004). To date, very few studies have reported that elevated 8-OHdG levels in follicular fluid are associated with poor oocyte quality (Tamura *et al.* 2008, Da Broi *et al.* 2016, Várnagy *et al.* 2020). The mean value for follicular 8-OHdG levels in this study was 22.40, which were much higher than those reported by Tamura *et al.* (2014)'s value of 5.7–6.6 µg/L and Várnagy *et al.* (2020)'s value of <12 µg/L but close to Da Broi *et al.* (2016)'s value of 17.22–23.19 µg/L. We found that follicular 8-OHdG levels in women with infertility problems (including cases with combined male and female infertility factors) (21.43 µg/L) were not significantly different from those in women without fertility problems (22.84 µg/L) with $P = 0.318$. The same results were obtained after cases when the combined male and female infertility factors were excluded. In seminal plasma, the mean 8-OHdG value was high (175.59 µg/L). Researchers have shown that elevated 8-OHdG levels in semen were associated with male infertility (Nakamura *et al.* 2002, Sakamoto *et al.* 2008, Cambi *et al.* 2013, Micillo *et al.* 2016, Tang *et al.* 2016, Vatannejad *et al.* 2017) and poor assisted reproductive technology (ART) outcome (Ahelik *et al.* 2015). Our 8-OHdG levels were much higher than 1.95 µg/L (Hammadeh *et al.* 2010); 36.4 µg/L reported by Sakamoto *et al.* (2008); 7.7 (subfertile) and 7.8 µg/L (infertile) (Nakamura *et al.* 2002); 0.004 µg/L (Amiri *et al.* 2011); and 0.08 (normal sperm count) vs 0.1 µg/L (poor motility sperm) (Vatannejad *et al.* 2017). Our seminal plasma 8-OHdG values represent all men who underwent IVF treatment. In our study, the seminal plasma 8-OHdG levels in men with fertility problems (including cases with both male and female infertility factors) were lower (167.21 µg/L) than those in men without fertility problems (186.72 µg/L), but the difference was of borderline significance ($P = 0.059$). The same results were obtained when cases with combined male and female infertility factors were excluded. Smoking has been consistently identified as a confounder of 8-OHdG (Pilger & Rudiger 2006), and 23.5% of the male partners were smokers. However, adjustment for the smoking factor did not alter the findings. An increase in 8-OHdG may not only reflect oxidative DNA damage but also indicate a decline in the DNA repair rate (Tchou & Grollman 1993, Halliwell 1998). An earlier study (Mecocci *et al.* 1998) observed that the deficit in DNA repair promotes the accumulation of 8-OHdG in peripheral cells, which could be a better indicator than urine, as urine mainly contains repaired products. However, not many studies have measured cellular 8-OHdG due to sample collection difficulty compared to the urine sample collection.

Catalase is an enzyme that reflects the follicular fluid's antioxidant efficiency, particularly during the early stages of folliculogenesis (Ruder *et al.* 2008, Gupta *et al.* 2011). CAT activity in the follicular fluid has been associated with fertilization and cleavage rates but not with pregnancy outcomes in women undergoing ART (Pasqualotto *et al.* 2009). Only a few studies have measured follicular fluid CAT activity, and they have reported that it is lower in older women (Carbone *et al.* 2003, Wdowiak 2015) and higher in obese women (Bausenwein *et al.* 2010). We cannot compare our values to these studies because they were adjusted for protein, except that of Wdowiak (2015). The authors reported CAT levels in follicular fluid of 12.82 U/mL in older women of 40–46 years old, significantly lower than in younger ones (18.91 U/mL). In the present study, CAT levels in the follicular fluid of women who were ≥40 years old (18.21 U/mL) were not significantly different from those in younger women (14.55 U/mL). This is probably because older women's sample size was much smaller than young women's sample size (42 vs 528). Further, in contrast to previous findings (Bausenwein *et al.* 2010), we found no significant difference in CAT levels between obese (BMI ≥ 30 kg/m^2) and non-obese women.

Since 1989, CAT activity in the semen has been recognized as an indicator of sperm function (Jeulin *et al.* 1989). CAT eliminates H_2O_2 by converting it to water and oxygen, thus, improving sperm motility (Baker *et al.* 1996). However, environmental and lifestyle factors, such as smoking, are known to reduce CAT activity and, subsequently, cause a decline in semen quality (Kumar *et al.* 2014). Unlike the follicular fluid findings, many studies have reported a reduction in the seminal plasma CAT activity among infertile men (Sharma & Agarwal 1996, Abd-Elmoaty *et al.* 2010, Agarwal *et al.* 2012*b*). In general, mean value for seminal CAT levels in the present study (10.132 U/mL) was much lower than those reported by Hajizadeh Maleki (2017) in all the four different groups of healthy men undergoing different levels and times of training and values reported by Khosrowbeygi (2007) for healthy vs infertile (22.58 vs 14.4 U/mL). However, in

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

the present study, we did not find significant differences in seminal CAT activities between normozoospermic and infertile men or between smokers and nonsmokers. Further, the CAT levels in follicular fluid and seminal plasma were both low. Thus, insufficient antioxidant levels may not protect against oxidative stress in both follicles and sperm, particularly during IVF treatment, as reported previously (Rakhit *et al.* 2013).

Elizur (2014) observed that H_2O_2 might be a potential marker for ovarian aging-related changes in follicular fluid's metabolic activity. H_2O_2 is considered a major contributor to oxidative stress because it oxidizes only a limited number of functional groups of biological molecules and is, therefore, only moderately reactive and permeable through membranes (Fujii & Tsunoda 2011). However, the findings reported by researchers are conflicting. While Gupta *et al.* (2011) found high levels of H_2O_2 in small bovine follicles that might have reflected an increase in ROS production during the earlier stages of folliculogenesis, Basini *et al.* (2008) observed reduced H_2O_2 levels in swine follicular fluids (which might indicate that oxidative stress does not affect follicle growth). Further, an experimental study found that high exposure to intracellular H_2O_2 activates myeloperoxidase and leads to oocyte quality deterioration (Khan *et al.* 2015). Our study is the first to provide data on the levels of H_2O_2 in human follicular fluid. In our study, the mean H_2O_2 value was 6.07 µg/L, and no significant difference was observed between infertile women and those without fertility problems. A study observed an increase in sperm motility in the presence of low H_2O_2 levels, which might, therefore, be a marker of antioxidant activity in sperm cells (Evdokimov *et al.* 2015). However, increased production of H_2O_2 can damage sperm function (Sanocka & Kurpisz 2004, Du Plessis *et al.* 2010). Elevated seminal plasma H_2O_2 levels have been reported in infertile men with severe inflammation (21.06 µg/L) and those without inflammation (21.94 µg/L) (Kullisaar *et al.* 2013). Both values were approximately two-fold higher than the mean value (10.66 µg/L) reported in this study. In the present study, although we found no significant difference in seminal H_2O_2 levels between normozoospermic and infertile men, other studies have shown that the seminal H_2O_2 levels in infertile men are five- to ten-fold higher than those in the control group (Kullisaar *et al.* 2013, Zandieh *et al.* 2018).

Follicular fluid represents a vital environment that contains steroid hormones, ROS, proteins, and antioxidants enzymes that are important for follicular development in the ovary (Agarwal *et al.* 2003, Ambekar *et al.* 2013, Freitas *et al.* 2017). Several studies found that higher follicular fluid TAC levels were associated with oocyte competence and successful pregnancy outcomes (Pasqualotto *et al.* 2004, Ruder *et al.* 2008, Bedaiwy *et al.* 2012, Kazemi *et al.* 2013). The mean follicular fluid TAC value in studied women (728.14 µM) was higher than those reported by Singh *et al.* (2013) for women with endometriosis (658.32 µM) but lower than those with tubal infertility (896.25 µM). In the present study, we observed significantly higher follicular fluid TAC among infertile women (785.41 µM) than the fertile ones (692.7 µM), but not different from those with other causes of female infertility. Infertile women, particularly those undergoing IVF treatment, tend to take multivitamins and supplements to strengthen their chance of getting pregnant (Ozkaya & Naziroglu 2010). This might explain the finding in our study population.

We observed no significant differences in seminal plasma TAC levels between normozoospermic (598.18 µM) and infertile men (570.52 µM). Both values were much lower than those reported for healthy male living in low (900 µM) and high (1200 µM) polluted areas (Bergamo *et al.* 2016). However, several studies have found lower TAC levels in the seminal plasma of infertile men (Pasqualotto *et al.* 2000, Giulini *et al.* 2009, Khosravi *et al.* 2014). An investigation established a diagnostic TAC cut-off value of 1947 µM in seminal plasma as a marker of oxidative stress among infertile men (Roychoudhury *et al.* 2016). Only one case in the present study had seminal TAC levels that were above this cut-off. A study found that smoking can lower TAC levels in infertile men's seminal plasma (Saleh *et al.* 2002). In the present study, smokers had slightly higher seminal TAC levels (603.59 µM) than nonsmokers (533.44 µM), but the difference was not statistically significant.

DNA damage profile in couples

A study recommended using human cumulus cells as a screening tool for female reproductive toxicants since they are readily available through IVF procedures, can be maintained using simple culture protocols, and show high potential to differentiate into granulosa cells with high predictability (Hughes *et al.* 1990). An earlier study by Sinko *et al.* (2005) detected DNA damage in cumulus cells related to smoking. The authors recommended using the comet assay to evaluate the effect of chemicals on the female reproductive system using cumulus cells because they play an essential role in oocyte maturation, ovulation, and fertilization. Here, we observed a high

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

degree of DNA damage in the cumulus cells, which might influence oocyte development's competence; however, this association has not been well established in the literature (Raman *et al.* 2001, Barcena *et al.* 2015, Tola *et al.* 2019). DNA damage in granulosa cells taken from women with infertility problems was not statistically different from that in women without infertility problems. We also observed that smoking did not cause DNA damage in the granulosa cells.

The comet assay has been recognized as a highly sensitive tool to assess sperm DNA damage, particularly in men with infertility (Schulte *et al.* 2010, Simon & Carrell 2013) and as a potential marker of the IVF/ICSI success (Nicopoullos *et al.* 2019). In the current study, the sperm cells showed evidence of DNA damage; however, unlike other studies, we found no differences in both parameters between normozoospermic and infertile men (Aydos *et al.* 2015, Ramzan *et al.* 2015, Fernandez-Encinas *et al.* 2016). Researchers observed that sperm dsDNA exhibits an increase in breaks with age (Singh *et al.* 2003, Das *et al.* 2013). However, no correlation was found in the present study between age and TM. Smoking is another factor that might induce DNA damage (Linschooten *et al.* 2011, Antoniassi *et al.* 2016), but in this study, we only noted that the DNA damage was significantly higher in male partners who smoked water pipes than nonsmokers ($P = 0.023$) but not in men who smoked cigarettes. Men who smoked both cigarettes and water pipes showed a significant increase in sperm cells DNA damage than nonsmokers ($P = 0.032$). A recent study showed a difference in DNA damage's susceptibility between the two sex chromosome (X and Y) spermatozoa (Shi *et al.* 2019).

Associations between oxidative stress/DNA damage biomarkers

In this study, biochemical markers, whether measured in women or their male partners, were inter-correlated. The higher the MDA levels in follicular fluid, the more was the DNA damage in the granulosa cells, as indicated by the TM values. These findings imply that follicles are more prone to oxidative stress and DNA damage. Granulosa cells play an essential role in protecting oocytes against oxidative stress-induced apoptosis by stimulating oocyte glutathione activity (Tatemoto *et al.* 2000); moreover, oocyte competence is associated with the DNA status of cumulus cells (Raman *et al.* 2001). Here, the follicular fluid 8-OHdG levels were inversely associated with DNA damage markers, but these two parameters were positively correlated in seminal plasma. This finding indicates that the imbalance between ROS production and antioxidant activity in follicular fluid and seminal plasma might differ, as corroborated by a previous study (Rahal *et al.* 2014). This could be partly explained by the positive association observed between DNA damage markers and follicular fluid TAC levels, which was not observed in seminal plasma; instead, the DNA damage markers were associated with H_2O_2 in seminal plasma. Sperm DNA damage is primarily a result of oxidative stress, which can be adequately managed with antioxidant therapy (Lewis *et al.* 2013, Agarwal *et al.* 2014).

The seminal plasma MDA and 8-OHdG levels were significantly correlated; this may indicate the effect of lipid peroxidation on DNA damage. A similar finding was reported by (Hosen *et al.* 2015), but (Nakamura *et al.* 2002) found contradictory findings. MDA can react with deoxyguanosine and deoxyadenosine in DNA to form adducts (Marnett 1999); this results in the formation of 8-OHdG, which is proportional to the lipid peroxidation level as reflected by MDA (Park & Floyd 1992). In follicular fluid, MDA was unexpectedly negatively associated with H_2O_2 and positively associated with TAC. As discussed earlier, both MDA and H_2O_2 are oxidative stress markers, while TAC represents the antioxidant defense system. Lipid peroxidation damage that is caused by ROS generated from H_2O_2 may depend on an equilibrium mechanism between the production and scavenging of ROS (Sharma *et al.* 2012). In seminal plasma, high ROS levels reflected by H_2O_2 were associated with an increase in lipid peroxidation and oxidative DNA damage, which are represented by MDA and 8-OHdG, respectively. Similar findings have been reported by other researchers (Colagar *et al.* 2009, Gharagozloo & Aitken 2011).

In follicular fluid, the higher the oxidative DNA damage indicated by 8-OHdG, the higher was the CAT activity observed. This confirms the antioxidant role of CAT in scavenging ROS, as discussed earlier. The high CAT activity in follicular fluid might explain the inverse association between H_2O_2 and 8-OHdG observed in this study. The positive correlation between CAT and TAC (Table 3) indicates that both scavenge ROSs generated from H_2O_2 in follicular fluid. This is probably because there is more demand for antioxidants in the follicular fluid of poorly developed oocytes (Singh *et al.* 2013, Revelli *et al.* 2017). Again, we observed that the more ROS generated in seminal plasma, the higher was the oxidative DNA damage. Unlike the observations in follicular fluid, in seminal plasma, CAT seems to have no role in reducing the ROS effect; this might reflect the antioxidant defense

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

system's inadequacy in seminal plasma. Such an imbalance between ROS production and antioxidant activity in the seminal plasma may impact spermatozoa and its overall fertilizing capacity (Agarwal *et al.* 2014).

The oxidative stress status in follicular fluid and seminal plasma may vary because they differ with regard to their extent to environmental exposure, ROS generation, and antioxidant capacity (Agarwal *et al.* 2003). Furthermore, oxidative stress markers are altered by smoking, diet, disease, genetic predispositions, and environmental factors (Kumar *et al.* 2014, Harlev *et al.* 2015, Bisht *et al.* 2017). However, these effects will not be discussed here because this is beyond the scope of this study.

Finally, we observed that CAT activity in follicular fluid increased significantly with urinary COT levels in women; this probably had a protective effect on cells from the damaging effects of ROS produced by smoking (Pasqualotto *et al.* 2008, Elshal *et al.* 2009). However, the seminal plasma's CAT activity was associated with male partners' urinary COT levels but with marginal significance. Our results showed that though none of the women in this study were smokers, COT was detected in 49.3% of follicular fluid samples. This was consequently associated with the induction of oxidative stress, as demonstrated by its association with H_2O_2 and TM. It has been reported that COT readily crosses the blood/follicle barrier, so this might explain our findings in follicular fluid (Paszkowski 1998). The COT present in follicular fluid might have impacted the quality of oocytes and their maturation. A study showed that COT interacts directly with and incorporates into the follicle cells and the developing oocyte, which has detrimental consequences after conception (Zenzes *et al.* 1997). Surprisingly, despite the high levels of the seminal plasma COT levels, it was only significantly associated with H_2O_2, which may negatively play a role in male fertility, as reported previously (Harlev *et al.* 2015).

Associations between biomarkers of oxidative stress/DNA damage and reproductive hormones

In the present study, we observed that higher levels of LH in women were associated with increased follicular fluid ROS generation, as represented by 8-OHdG. Twenty-nine women (4.9%) had LH above the upper KFSH&RC reference limit of 12.6 IU/L. Researchers have warned that the early surges in LH usually prevent the effective induction of multiple follicular maturation patterns (Al-Inany *et al.* 2016). On the other hand, lower LH has been associated with greater DNA damage in cumulus cells, as indicated by the TM level. LH induces ROS generation to modulate oocyte maturation, ovarian steroidogenesis, corpus luteal function, and luteolysis, which are generally maintained by the well-balanced antioxidant system (Kala *et al.* 2017). In this study, 138 (23.4%) women had LH levels that were lower than the KFSH&RC reference limit of 2.4 U/L, which is indicative of poor reproductive performance (Peñarrubia *et al.* 2003). This might have impaired the antioxidant system and led to ROS overproduction and subsequent DNA damage.

The higher ROS generation, in the form of H_2O_2, was associated with a reduction in serum TSH levels in women. Low TSH levels are indicative of hyperthyroidism (Obuobie & Jones 2003). Only five women had TSH levels lower than the reference limit of 0.27 mU/L, which indicates overactive thyroid function, and 73 had TSH levels above the upper limit of 4.2 mU/L, which suggests hypothyroidism. Both hyper- and hypothyroidism have been linked to female infertility (American Society for Reproductive Medicine 2015, Mintziori *et al.* 2016). Additionally, the association between oxidative stress and excess or low TSH levels is well documented (Mancini *et al.* 2016). It has been reported that hyperthyroidism increases oxidative stress, whereas hypothyroidism decreases ROS production and antioxidant activity (Villanueva *et al.* 2013).

The DNA damage marker TM was inversely associated with higher E_2 levels in women. Low E_2 reflects poor ovarian response after using standard stimulation IVF protocols (Tarlatzis *et al.* 2003, Jirge 2016). A study by Kalemba-Drozdz (2015) found that E_2 is a naturally occurring antioxidant that may play a protective role during pregnancy. The authors found that E_2 was negatively associated with oxidative DNA damage. In this study, 32.4% of women had lower than normal E_2 levels (lower than the KFSH&RC reference limit of 46 pmol/L). Based on these findings, it can be assumed that the lower the E_2 level, the higher is the DNA damage in cumulus cells.

The higher the seminal plasma MDA levels in male partners, the lesser were the serum FSH levels. FSH plays an essential role in maintaining male reproductive functions (Ulloa-Aguirre & Lira-Albarran 2016). Further, increased ROS generation in seminal plasma potentially affects male infertility (Sabeti *et al.* 2016). Here, 9.3% of the men had FSH levels lower than the KFSH&RC reference limit of 1.5 IU/l. ROS generated by alcohol, smoking, or environmental factors might affect FSH synthesis (Oremosu & Akang 2015, Aprioku & Ugwu 2016, Zubair

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

et al. 2017). Research has shown that ROS affects FSH's secretion via its effect on the gonadotropin-releasing hormone (Terasaka *et al.* 2017). This mechanism could not be corroborated in the present study.

Increased levels of 8-OHdG and MDA in seminal plasma have been associated with male infertility (Huang *et al.* 2018). We observed that higher seminal plasma levels of MDA and 8-OHdG were associated with elevated serum T levels in the male partners. Although oxidative stress can result in Leydig cell dysfunction or apoptosis and diminished T production (Dabaja *et al.* 2013), experimental studies have reported that T can induce oxidative stress on account of its pro-oxidant properties (Alonso-Alvarez *et al.* 2007) and act as an antioxidant by protecting sperm and other testicular cells from ROS damage (Darbandi *et al.* 2018). As T plays a vital role in the initiation and maintenance of spermatogenesis (Gudeloglu & Parekattil 2013), abnormal T levels may directly impact male fertility.

We want to acknowledge a major limitation of our study. As reported previously, the levels of reproductive hormones may vary within an individual over time, and not all patients were assessed on their initial visit to the clinic (Brambilla *et al.* 2009).

Impact of oxidative stress and DNA damage on IVF outcomes

The present study showed that high CAT levels in follicular fluid might reduce the risk of poor fertilization rate (≤50%) and unsuccessful live birth by 48 and 41%, respectively. Pasqualotto *et al.* (2009) observed a correlation between follicular fluid CAT levels and fertilization rate, but not with pregnancy rate. Here, after the multivariate log-binomial regression models were adjusted for the hormonal factors FSH, LH, and E_2, only the association between elevated follicular fluid CAT levels and low risk of poor fertilization remained significant. However, when the models controlled for hormonal factors TSH and Prl, high CAT levels in follicular fluid were still associated with a reduced risk of both poor fertilization rate (≤50%) and unsuccessful live birth. In contrast to the findings of Oyawoye *et al.* (2003) and Varnagy *et al.* (2018), we also observed an association between high levels of follicular TAC and a low probability of poor fertilization rate.

It has been suggested that sex hormones have a potential role in regulating the synthesis and activity of antioxidants (Mancini *et al.* 2010) or in regulating antioxidant gene expression (Bellanti *et al.* 2013). Although several studies have investigated the impact of oxidative stress in follicular fluid on IVF outcomes, it is hard to draw a definite conclusion because of conflicting results resulting from the use of various markers and IVF outcomes such as live birth, oocyte quality, fertilization rate, and pregnancy rate (Askoxylaki *et al.* 2013). For example, some studies have reported associations between high follicular fluid levels of TAC and pregnancy and increase in follicle size (Gupta *et al.* 2011, Bedaiwy *et al.* 2012), between lipid peroxidation and pregnancy rate (Pasqualotto *et al.* 2009), between 8-OHdG and oocyte quality (Tamura *et al.* 2008), between superoxide dismutase and the quality of embryos on the second day of development (Wdowiak 2015), and between MDA and oocyte retrieval (Thaker *et al.* 2020). From all these findings, it seems that ROS affects embryo development at different stages. Our results indicate that the CAT and TAC activities observed in the follicular fluid samples probably played a role in neutralizing ROS production. Therefore, as reported in a previous study, CAT and TAC could be potential markers of mature follicles, leading to the growth of high-quality oocytes (Gupta *et al.* 2011).

Study limitations and strengths

The study has several limitations to consider when interpreting the results. First, the studied population was recruited from the IVF clinic, thereby restricting the generalization of our findings to the general population. Secondly, the possibility of uncontrolled potential confounding variables that might be related to the oxidative stress and/or the outcomes (IVF endpoints). Thirdly, self-reported variables might have introduced a bias in the results. Fourthly, the use of ELISA assays to measure oxidative stress biomarkers that might be less accurate than mass spectrometry techniques. Fifthly, no internal quality controls were provided by the ELISA manufacturers to evaluate the intra-and -inter-assay precision. Sixthly, given ROS's highly reactive nature, the stability of H_2O_2 might be affected after a lengthy storage period. However, the samples were processed on time that hopefully make the change constant. Despite these limitations, our study has many strengths: (1) large sample size, (2) prospective design, (3) measurement of several oxidative stress biomarkers in couples, (4) being conducted in an IVF setting, giving us access to various endpoints, and (5) numerous confounding variables concerning demographic, socioeconomic, lifestyle, and health collected from each couple.

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-20-0062

Conclusions

The present study results show that oxidative stress and DNA damage biomarkers in couples undergoing IVF were associated with several reproductive hormones and smoking status. The findings indicate ROS's disruptive effect on hormones and the consequent adverse effects on male and female reproductive functions. In this regard, our results demonstrated that elevated levels of CAT in follicular fluid reduced the risk of poor fertilization rate (≥50%) and unsuccessful live birth. Further, higher levels of follicular fluid TAC were found to reduce the risk of poor fertilization rate but only after the data were adjusted for hormonal factors. This indicates the potential role of these hormones in regulating antioxidant behavior. In conclusion, higher antioxidant activity in follicular fluid might have a positive impact on specific IVF outcomes. Additionally, biomarkers of oxidative stress and DNA damage might have potential applications in evaluating IVF patients' clinical characteristics.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This work was supported by King Abdulaziz City for Science and Technology for funding this research (grant number ARP-34-27, 2013); and King Faisal Specialist Hospital and Research Centre (RAC#2132024, 2013).

Data availability
The data that has been used is confidential.

Author contribution statement
Iman Al-Saleh was involved in designing/writingpProject management and supervision. Coskun Serdar was involved in IVF procedures/sample collection and parameters. Reem Al-Rouqi, Tahreer Al-Rajoudi, Chafica Eltabache, and Mai Abduljabbar were involved in Methodology/validation. Saad Al-Hassan was involved in clinical assessment and selection of IVF couples.

References

Abd-Elmoaty MA, Saleh R, Sharma R & Agarwal A 2010 Increased levels of oxidants and reduced antioxidants in semen of infertile men with varicocele. *Fertility and Sterility* **94** 1531–1534. (https://doi.org/10.1016/j.fertnstert.2009.12.039)

Agarwal A & Allamaneni SS 2011 Free radicals and male reproduction. *Journal of the Indian Medical Association* **109** 184–187.

Agarwal A & Sekhon LH 2011 Oxidative stress and antioxidants for idiopathic oligoasthenoteratospermia: is it justified? *Indian Journal of Urology* **27** 74–85. (https://doi.org/10.4103/0970-1591.78437)

Agarwal A, Saleh RA & Bedaiwy MA 2003 Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertility and Sterility* **79** 829–843. (https://doi.org/10.1016/s0015-0282(02)04948-8)

Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A & Gupta S 2012*a* The effects of oxidative stress on female reproduction: a review. *Reproductive Biology and Endocrinology* **10** 49–49. (https://doi.org/10.1186/1477-7827-10-49)

Agarwal A, Hamada A & Esteves SC 2012*b* Insight into oxidative stress in varicocele-associated male infertility: part 1. *Nature Reviews: Urology* **9** 678–690. (https://doi.org/10.1038/nrurol.2012.197)

Agarwal A, Virk G, Ong C & Du Plessis SS 2014 Effect of oxidative stress on male reproduction. *World Journal of Men's Health* **32** 1–17. (https://doi.org/10.5534/wjmh.2014.32.1.1)

Agarwal A, Roychoudhury S, Bjugstad KB & Cho CL 2016 Oxidation-reduction potential of semen: what is its role in the treatment of male infertility? *Therapeutic Advances in Urology* **8** 302–318. (https://doi.org/10.1177/1756287216652779)

Ahelik A, Mandar R, Korrovits P, Karits P, Talving E, Rosenstein K, Jaagura M, Salumets A & Kullisaar T 2015 Systemic oxidative stress could predict assisted reproductive technique outcome. *Journal of Assisted Reproduction and Genetics* **32** 699–704.

Al-Inany HG, Youssef MA, Ayeleke RO, Brown J, Lam WS & Broekmans FJ 2016 Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. *Cochrane Database of Systematic Reviews* **4** CD001750. (https://doi.org/10.1002/14651858.CD001750.pub4)

Alonso-Alvarez C, Bertrand S, Faivre B, Chastel O & Sorci G 2007 Testosterone and oxidative stress: the oxidation handicap hypothesis. *Proceedings: Biological Sciences* **274** 819–825. (https://doi.org/10.1098/rspb.2006.3764)

Al-Saleh I, Billedo G & Shinwari N 2007 Serum malonyldialdehyde levels in Saudi population and its relationship to a number of factors such as age, gender, antioxidant levels and health status. *Trace Elements and Electrolytes* **24** 103–111. (https://doi.org/10.5414/TEP24103)

Ambekar AS, Nirujogi RS, Srikanth SM, Chavan S, Kelkar DS, Hinduja I, Zaveri K, Prasad TS, Harsha HC, Pandey A, et al. 2013 Proteomic analysis of human follicular fluid: a new perspective towards understanding folliculogenesis. *Journal of Proteomics* **87** 68–77. (https://doi.org/10.1016/j.jprot.2013.05.017)

American Society for Reproductive Medicine 2015 Subclinical hypothyroidism in the infertile female population: a guideline. *Fertility and Sterility* **104** 545–553. (https://doi.org/10.1016/j.fertnstert.2015.05.028)

Amiri I, Karimi J, Piri H, Goodarzi MT, Tavilani H, Khodadadi I & Ghorbani M 2011 Association between nitric oxide and 8-hydroxydeoxyguanosine levels in semen of diabetic men. *Systems Biology in Reproductive Medicine* **57** 292–295. (https://doi.org/10.3109/19396368.2011.621508)

Antoniassi MP, Intasqui P, Camargo M, Zylbersztejn DS, Carvalho VM, Cardozo KH & Bertolla RP 2016 Analysis of the functional aspects and seminal plasma proteomic profile of sperm from smokers. *BJU International* **118** 814–822. (https://doi.org/10.1111/bju.13539)

Aprioku JS & Ugwu TC 2016 Tobacco smoke exposure induces irreversible alteration of testicular function in prepubertal rats. *Journal of Basic and Clinical Physiology and Pharmacology* **27** 577–584. (https://doi.org/10.1515/jbcpp-2015-0153)

Artimani T, Karimi J, Mehdizadeh M, Yavangi M, Khanlarzadeh E, Ghorbani M, Asadi S & Kheiripour N

2018 Evaluation of pro-oxidant-antioxidant balance (PAB) and its association with inflammatory cytokines in polycystic ovary syndrome (PCOS). *Gynecological Endocrinology* **34**148–152. (https://doi.org/10.1080/09513590.2017.1371691)

Asadi N, Bahmani M, Kheradmand A & Rafieian-Kopaei M 2017 The impact of oxidative stress on testicular function and the role of antioxidants in improving it: a review. *Journal of Clinical and Diagnostic Research* **11** IE01–IE05. (https://doi.org/10.7860/JCDR/2017/23927.9886)

Askoxylaki M, Siristatidis C, Chrelias C, Vogiatzi P, Creatsa M, Salamalekis G, Vrantza T, Vrachnis N & Kassanos D 2013 Reactive oxygen species in the follicular fluid of subfertile women undergoing in vitro fertilization: a short narrative review. *Journal of Endocrinological Investigation* **36** 1117–1120. (https://doi.org/10.3275/9097)

Atig F, Kerkeni A, Saad A & Ajina M 2017 Effects of reduced seminal enzymatic antioxidants on sperm DNA fragmentation and semen quality of Tunisian infertile men. *Journal of Assisted Reproduction and Genetics* **34** 373–381. (https://doi.org/10.1007/s10815-013-9936-x)

Avila J, Gonzalez-Fernandez R, Rotoli D, Hernandez J & Palumbo A 2016 Oxidative stress in granulosa-lutein cells from in vitro fertilization patients. *Reproductive Sciences* **23** 1656–1661. (https://doi.org/10.1177/1933719116674077)

Aydos OS, Yukselten Y, Kaplan F, Sunguroglu A & Aydos K 2015 Analysis of the correlation between sperm DNA integrity and conventional semen parameters in infertile men. *Turkish Journal of Urology* **41** 191–197. (https://doi.org/10.5152/tud.2015.98475)

Baker HW, Brindle J, Irvine DS & Aitken RJ 1996 Protective effect of antioxidants on the impairment of sperm motility by activated polymorphonuclear leukocytes. *Fertility and Sterility* **65** 411–419. (https://doi.org/10.1016/s0015-0282(16)58109-6)

Balhara YP, Jain R, Sundar AS & Sagar R 2012 Use of cotinine urinalysis to verify self-reported tobacco use among male psychiatric out-patients. *Lung India* **29** 217–220. (https://doi.org/10.4103/0970-2113.99102)

Barcena P, López-Fernández C, García-Ochoa C, Obradors A, Vernaeve V, Gosálvez J & Vassena R 2015 Detection of DNA damage in cumulus cells using a chromatin dispersion assay. *Systems Biology in Reproductive Medicine* **61** 277–285. (https://doi.org/10.3109/19396368.2015.1063739)

Barros AJD & Hirakata VN 2003 Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. *BMC Medical Research Methodology* **3** 21. (https://doi.org/10.1186/1471-2288-3-21)

Basini G, Simona B, Santini SE & Grasselli F 2008 Reactive oxygen species and anti-oxidant defences in swine follicular fluids. *Reproduction, Fertility, and Development* **20** 269–274. (https://doi.org/10.1071/rd07147)

Bausenwein J, Serke H, Eberle K, Hirrlinger J, Jogschies P, Hmeidan FA, Blumenauer V & Spanel-Borowski K 2010 Elevated levels of oxidized low-density lipoprotein and of catalase activity in follicular fluid of obese women. *Molecular Human Reproduction* **16** 117–124. (https://doi.org/10.1093/molehr/gap078)

Bedaiwy MA, Elnashar SA, Goldberg JM, Sharma R, Mascha EJ, Arrigain S, Agarwal A & Falcone T 2012 Effect of follicular fluid oxidative stress parameters on intracytoplasmic sperm injection outcome. *Gynecological Endocrinology* **28** 51–55. (https://doi.org/10.3109/09513590.2011.579652)

Bellanti F, Matteo M, Rollo T, De Rosario F, Greco P, Vendemiale G & Serviddio G 2013 Sex hormones modulate circulating antioxidant enzymes: impact of estrogen therapy. *Redox Biology* **1** 340–346. (https://doi.org/10.1016/j.redox.2013.05.003)

Benedict MD, Missmer SA, Vahratian A, Berry KF, Vitonis AF, Cramer DW & Meeker JD 2011 Secondhand tobacco smoke exposure is associated with increased risk of failed implantation and reduced IVF success. *Human Reproduction* **26** 2525–2531. (https://doi.org/10.1093/humrep/der226)

Bergamo P, Volpe MG, Lorenzetti S, Mantovani A, Notari T, Cocca E, Cerullo S, Di Stasio M, Cerino P & Montano L 2016 Human semen as an early, sensitive biomarker of highly polluted living environment in healthy men: a pilot biomonitoring study on trace elements in blood and semen and their relationship with sperm quality and RedOx status. *Reproductive Toxicology* **66** 1–9. (https://doi.org/10.1016/j.reprotox.2016.07.018)

Bisht S, Faiq M, Tolahunase M & Dada R 2017 Oxidative stress and male infertility. *Nature Reviews: Urology* **14** 470–485. (https://doi.org/10.1038/nrurol.2017.69)

Black CN, Bot M, Scheffer PG & Penninx BW 2016 Sociodemographic and lifestyle determinants of plasma oxidative stress markers 8-OHdG and F2-isoprostanes and associations with metabolic syndrome. *Oxidative Medicine and Cellular Longevity* **2016** 7530820. (https://doi.org/10.1155/2016/7530820)

Brambilla DJ, Matsumoto AM, Araujo AB & Mckinlay JB 2009 The effect of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men. *Journal of Clinical Endocrinology and Metabolism* **94** 907–913. (https://doi.org/10.1210/jc.2008-1902)

Cambi M, Tamburrino L, Marchiani S, Olivito B, Azzari C, Forti G, Baldi E & Muratori M 2013 Development of a specific method to evaluate 8-hydroxy, 2-deoxyguanosine in sperm nuclei: relationship with semen quality in a cohort of 94 subjects. *Reproduction* **145** 227–235. (https://doi.org/10.1530/REP-12-0404)

Carbone MC, Tatone C, Delle Monache S, Marci R, Caserta D, Colonna R & Amicarelli F 2003 Antioxidant enzymatic defences in human follicular fluid: characterization and age-dependent changes. *Molecular Human Reproduction* **9** 639–643. (https://doi.org/10.1093/molehr/gag090)

Chari MG & Colagar AH 2011 Seminal plasma lipid peroxidation, total antioxidant capacity, and cigarette smoking in asthenoteratospermic men. *Journal of Men's Health* **8** 43–49. (https://doi.org/10.1016/j.jomh.2010.09.230)

Colagar AH, Pouramir M, Marzony ET & Jorsaraei SGA 2009 Relationship between seminal malondialdehyde levels and sperm quality in fertile and infertile men. *Brazilian Archives of Biology and Technology* **52** 1387–1392. (https://doi.org/10.1590/S1516-89132009000600010)

Collins AR 2015 The comet assay: a heavenly method! *Mutagenesis* **30** 1–4. (https://doi.org/10.1093/mutage/geu079)

Collodel G, Moretti E, Micheli L, Menchiari A, Moltoni L & Cerretani D 2015 Semen characteristics and malondialdehyde levels in men with different reproductive problems. *Andrology* **3** 280–286. (https://doi.org/10.1111/andr.297)

Da Broi MG, De Albuquerque FO, De Andrade AZ, Cardoso RL, Jordao Junior AA & Navarro PA 2016 Increased concentration of 8-hydroxy-2′-deoxyguanosine in follicular fluid of infertile women with endometriosis. *Cell and Tissue Research* **366** 231–242. (https://doi.org/10.1007/s00441-016-2428-4)

Da Silva J 2016 DNA damage induced by occupational and environmental exposure to miscellaneous chemicals. *Mutation Research* **770** 170–182. (https://doi.org/10.1016/j.mrrev.2016.02.002)

Dabaja A, Wosnitzer M & Goldstein M 2013 Varicocele and hypogonadism. *Current Urology Reports* **14** 309–314. (https://doi.org/10.1007/s11934-013-0339-4)

Dabrowska N & Wiczkowski A 2017 Analytics of oxidative stress markers in the early diagnosis of oxygen DNA damage. *Advances in Clinical and Experimental Medicine* **26** 155–166. (https://doi.org/10.17219/acem/43272)

Darbandi M, Darbandi S, Agarwal A, Sengupta P, Durairajanayagam D, Henkel R & Sadeghi MR 2018 Reactive oxygen species and male reproductive hormones. *Reproductive Biology*

and Endocrinology **16** 87–87. (https://doi.org/10.1186/s12958-018-0406-2)

Das M, Al-Hathal N, San-Gabriel M, Phillips S, Kadoch IJ, Bissonnette F, Holzer H & Zini A 2013 High prevalence of isolated sperm DNA damage in infertile men with advanced paternal age. *Journal of Assisted Reproduction and Genetics* **30** 843–848. (https://doi.org/10.1007/s10815-013-0015-0)

Dattilo M, D'Amato G, Caroppo E & Ménézo Y 2016 Improvement of gamete quality by stimulating and feeding the endogenous antioxidant system: mechanisms, clinical results, insights on gene-environment interactions and the role of diet. *Journal of Assisted Reproduction and Genetics* **33** 1633–1648. (https://doi.org/10.1007/s10815-016-0767-4)

De Lima CB, Cordeiro FB, Camargo M, Zylbersztejn DS, Cedenho AP, Bertolla RP & Lo Turco EG 2017 Follicular fluid lipid peroxidation levels in women with endometriosis during controlled ovarian hyperstimulation. *Human Fertility* **20** 48–54. (https://doi.org/10.1080/14647273.2016.1246753)

Devine PJ, Perreault SD & Luderer U 2012 Roles of reactive oxygen species and antioxidants in ovarian toxicity. *Biology of Reproduction* **86** 27. (https://doi.org/10.1095/biolreprod.111.095224)

Du Plessis SS, Mcallister DA, Luu A, Savia J, Agarwal A & Lampiao F 2010 Effects of H2O2 exposure on human sperm motility parameters, reactive oxygen species levels and nitric oxide levels. *Andrologia* **42** 206–210. (https://doi.org/10.1111/j.1439-0272.2009.00980.x)

Einaudi L, Courbiere B, Tassistro V, Prevot C, Sari-Minodier I, Orsiere T & Perrin J 2014 In vivo exposure to benzo(a)pyrene induces significant DNA damage in mouse oocytes and cumulus cells. *Human Reproduction* **29** 548–554. (https://doi.org/10.1093/humrep/det439)

Elizur SE, Lebovitz O,Orvieto R,Dor J& Zan-Bar T 2014 Reactive oxygen species in follicular fluid may serve as biochemical markers to determine ovarian aging and follicular metabolic age. *Gynecological Endocrinology* **30** 705–707. (https://doi.org/10.3109/09513590.2014.924100)

Elshal MF, El-Sayed IH, Elsaied MA, El-Masry SA & Kumosani TA 2009 Sperm head defects and disturbances in spermatozoal chromatin and DNA integrities in idiopathic infertile subjects: association with cigarette smoking. *Clinical Biochemistry* **42** 589–594. (https://doi.org/10.1016/j.clinbiochem.2008.11.012)

Evans MD, Dizdaroglu M & Cooke MS 2004 Oxidative DNA damage and disease: induction, repair and significance. *Mutation Research* **567** 1–61. (https://doi.org/10.1016/j.mrrev.2003.11.001)

Evdokimov VV, Barinova KV, Turovetskii VB, Muronetz VI & Schmalhausen EV 2015 Low concentrations of hydrogen peroxide activate the antioxidant defense system in human sperm cells. *Biochemistry: Biokhimiia* **80** 1178–1185. (https://doi.org/10.1134/S0006297915090084)

Fatemi F, Mohammadzadeh A, Sadeghi MR, Akhondi MM, Mohammadmoradi S, Kamali K, Lackpour N, Jouhari S, Zafadoust S, Mokhtar S, et al. 2017 Role of vitamin E and D3 supplementation in intra-cytoplasmic sperm injection outcomes of women with polycystic ovarian syndrome: a double blinded randomized placebo-controlled trial. *Clinical Nutrition ESPEN* **18** 23–30. (https://doi.org/10.1016/j.clnesp.2017.01.002)

Fernandez-Encinas A, Garcia-Peiro A, Ribas-Maynou J, Abad C, Amengual MJ, Navarro J & Benet J 2016 Characterization of nuclease activity in human seminal plasma and its relationship to semen parameters, sperm DNA fragmentation and male infertility. *Journal of Urology* **195** 213–219. (https://doi.org/10.1016/j.juro.2015.07.089)

Freinbichler W, Colivicchi MA, Stefanini C, Bianchi L, Ballini C, Misini B, Weinberger P, Linert W, Vareslija D, Tipton KF, et al. 2011 Highly reactive oxygen species: detection, formation, and possible functions. *Cellular and Molecular Life Sciences* **68** 2067–2079. (https://doi.org/10.1007/s00018-011-0682-x)

Freitas C, Neto AC, Matos L, Silva E, Ribeiro Â, Silva-Carvalho JL & Almeida H 2017 Follicular fluid redox involvement for ovarian follicle growth. *Journal of Ovarian Research* **10** 44. (https://doi.org/10.1186/s13048-017-0342-3)

Fuentes A, Muñoz A, Barnhart K, Argüello B, Díaz M & Pommer R 2010 Recent cigarette smoking and assisted reproductive technologies outcome. *Fertility and Sterility* **93** 89–95. (https://doi.org/10.1016/j.fertnstert.2008.09.073)

Fujii J & Tsunoda S 2011 Redox regulation of fertilisation and the spermatogenic process. *Asian Journal of Andrology* **13** 420–423. (https://doi.org/10.1038/aja.2011.10)

Gandhi J, Hernandez RJ, Chen A, Smith NL, Sheynkin YR, Joshi G & Khan SA 2017 Impaired hypothalamic-pituitary-testicular axis activity, spermatogenesis, and sperm function promote infertility in males with lead poisoning. *Zygote* **25** 103–110. (https://doi.org/10.1017/S0967199417000028)

Gharagozloo P & Aitken RJ 2011 The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy. *Human Reproduction* **26** 1628–1640. (https://doi.org/10.1093/humrep/der132)

Giulini S, Sblendorio V, Xella S, La Marca A, Palmieri B & Volpe A 2009 Seminal plasma total antioxidant capacity and semen parameters in patients with varicocele. *Reproductive Biomedicine Online* **18** 617–621. (https://doi.org/10.1016/s1472-6483(10)60004-1)

Greco F, Perrin J, Auffan M, Tassistro V, Orsiere T & Courbiere B 2015 A new approach for the oocyte genotoxicity assay: adaptation of comet assay on mouse cumulus-oocyte complexes. *Laboratory Animals* **49** 251–254. (https://doi.org/10.1177/0023677214567136)

Grotto D, Maria LS, Valentini J, Paniz C, Schmitt G, Garcia SC, Pomblum VJ, Rocha JBT & Farina M 2009 Importance of the lipid peroxidation biomarkers and methodological aspects FOR malondialdehyde quantification. *Química Nova* **32** 169–174. (https://doi.org/10.1590/S0100-40422009000100032)

Gudeloglu A & Parekattil SJ 2013 Update in the evaluation of the azoospermic male. *Clinics* **68** (Supplement 1) 27–34. (https://doi.org/10.6061/clinics/2013(sup01)04)

Guerriero G, Trocchia S, Abdel-Gawad FK & Ciarcia G 2014 Roles of reactive oxygen species in the spermatogenesis regulation. *Frontiers in Endocrinology* **5** 56. (https://doi.org/10.3389/fendo.2014.00056)

Gunasekarana V, Raj GV & Chand P 2015 A comprehensive review on clinical applications of comet assay. *Journal of Clinical and Diagnostic Research* **9** GE01–GE05. (https://doi.org/10.7860/JCDR/2015/12062.5622), **Ge**01–05.

Gupta S, Choi A, Yu HY, Czerniak SM, Holick EA, Paolella LJ, Agarwal A & Combelles CM 2011 Fluctuations in total antioxidant capacity, catalase activity and hydrogen peroxide levels of follicular fluid during bovine folliculogenesis. *Reproduction, Fertility, and Development* **23** 673–680. (https://doi.org/10.1071/RD10270)

Hajizadeh Maleki B, Tartibian B& Chehrazi M 2017 The effects of three different exercise modalities on markers of male reproduction in healthy subjects: a randomized controlled trial. *Reproduction* **153** 157–174. (https://doi.org/10.1530/REP-16-0318)

Halliwell B 1998 Can oxidative DNA damage be used as a biomarker of cancer risk in humans? Problems, resolutions and preliminary results from nutritional supplementation studies. *Free Radical Research* **29** 469–486. (https://doi.org/10.1080/10715769800300531)

Hammadeh ME, Hamad MF, Montenarh M & Fischer-Hammadeh C 2010 Protamine contents and P1/P2 ratio in human spermatozoa from smokers and non-smokers. *Human Reproduction* **25** 2708–2720. (https://doi.org/10.1093/humrep/deq226)

Harlev A, Agarwal A, Gunes SO, Shetty A & Du Plessis SS 2015 Smoking and male infertility: an evidence-based review. *World Journal of Men's Health* **33** 143–160. (https://doi.org/10.5534/wjmh.2015.33.3.143)

Hartmann A & Speit G 1997 The contribution of cytotoxicity to DNA-effects in the single cell gel test (comet assay). *Toxicology Letters* **90** 183–188. (https://doi.org/10.1016/s0378-4274(96)03847-7)

Hosen MB, Islam MR, Begum F, Kabir Y & Howlader MZ 2015 Oxidative stress induced sperm DNA damage, a possible reason for male infertility. *Iranian Journal of Reproductive Medicine* **13** 525–532.

Huang C, Cao X, Pang D, Li C, Luo Q, Zou Y, Feng B, Li L, Cheng A & Chen Z 2018 Is male infertility associated with increased oxidative stress in seminal plasma? A-meta analysis. *Oncotarget* **9** 24494–24513. (https://doi.org/10.18632/oncotarget.25075)

Hughes SF, Haney AF & Hughes Jr CL 1990 Use of human cumulus granulosa cells for in vitro screening of reproductive toxicants. *Reproductive Toxicology* **4** 11–15. (https://doi.org/10.1016/0890-6238(90)90073-5)

Jarvis MJ, Tunstall-Pedoe H, Feyerabend C, Vesey C & Saloojee Y 1987 Comparison of tests used to distinguish smokers from nonsmokers. *American Journal of Public Health* **77** 1435–1438. (https://doi.org/10.2105/ajph.77.11.1435)

Jeulin C, Soufir JC, Weber P, Laval-Martin D & Calvayrac R 1989 Catalase activity in human spermatozoa and seminal plasma. *Gamete Research* **24** 185–196. (https://doi.org/10.1002/mrd.1120240206)

Jirge PR 2016 Poor ovarian reserve. *Journal of Human Reproductive Sciences* **9** 63–69. (https://doi.org/10.4103/0974-1208.183514)

Josarayi GA, Mohammad-Hasani A, Aftabi Y, Moudi E & Hosseinzadeh Colagar A 2017 The AhRR-c.565C>G transversion may increase total antioxidant capacity levels of the seminal plasma in infertile men. *Environmental Science and Pollution Research International* **24** 17428–17435. (https://doi.org/10.1007/s11356-017-9356-z)

Jozwik M, Wolczynski S,Jozwik M& Szamatowicz M 1999 Oxidative stress markers in preovulatory follicular fluid in humans. *Molecular Human Reproduction* **5** 409–413. (https://doi.org/10.1093/molehr/5.5.409)

Kala M, Shaikh MV & Nivsarkar M 2017 Equilibrium between anti-oxidants and reactive oxygen species: a requisite for oocyte development and maturation. *Reproductive Medicine and Biology* **16** 28–35. (https://doi.org/10.1002/rmb2.12013)

Kalemba-Drozdz M 2015 The interaction between air pollution and diet does not influence the DNA damage in lymphocytes of pregnant women. *Environmental Research* **136** 295–299. (https://doi.org/10.1016/j.envres.2014.10.020)

Kazemi A, Ramezanzadeh F, Nasr-Esfahani MH, Saboor Yaraghi AA & Ahmadi M 2013 Does dietary fat intake influence oocyte competence and embryo quality by inducing oxidative stress in follicular fluid? *Iranian Journal of Reproductive Medicine* **11** 1005–1012.

Kazemi A, Ramezanzadeh F, Nasr Esfahani MH, Saboor-Yaraghi AA, Nejat SN & Rahimi-Foroshani A 2014 Relationship between energy expenditure related factors and oxidative stress in follicular fluid. *International Journal of Fertility and Sterility* **8** 175–182.

Kazemi A, Ramezanzadeh F& Nasr-Esfahani MH 2015 The relations between dietary antioxidant vitamins intake and oxidative stress in follicular fluid and ART outcomes. *Iranian Journal of Reproductive Medicine* **13** 533–540.

Khan SN, Shaeib F, Najafi T, Kavdia M, Gonik B, Saed GM, Goud PT & Abu-Soud HM 2015 Diffused intra-oocyte hydrogen peroxide activates myeloperoxidase and deteriorates oocyte quality. *PLoS ONE* **10** e0132388. (https://doi.org/10.1371/journal.pone.0132388)

Khosravi F, Valojerdi MR, Amanlou M, Karimian L & Abolhassani F 2014 Relationship of seminal reactive nitrogen and oxygen species and total antioxidant capacity with sperm DNA fragmentation in infertile couples with normal and abnormal sperm parameters. *Andrologia* **46** 17–23. (https://doi.org/10.1111/and.12034)

Khosrowbeygi A & Zarghami N 2007 Levels of oxidative stress biomarkers in seminal plasma and their relationship with seminal parameters. *BMC Clinical Pathology* **7** 6–6. (https://doi.org/10.1186/1472-6890-7-6)

Kullisaar T, Turk S, Kilk K, Ausmees K, Punab M & Mändar R 2013 Increased levels of hydrogen peroxide and nitric oxide in male partners of infertile couples. *Andrology* **1** 850–858. (https://doi.org/10.1111/j.2047-2927.2013.00123.x)

Kumar S, Murarka S, Mishra VV & Gautam AK 2014 Environmental and lifestyle factors in deterioration of male reproductive health. *Indian Journal of Medical Research* **140** (Supplement) S29–S35.

Kumar S, Mishra V, Thaker R, Gor M, Perumal S, Joshi P, Sheth H, Shaikh I, Gautam AK & Verma Y 2018 Role of environmental factors & oxidative stress with respect to in vitro fertilization outcome. *Indian Journal of Medical Research* **148** S125–S133. (https://doi.org/10.4103/ijmr.IJMR_1864_17)

Kumaravel TS, Vilhar B, Faux SP & Jha AN 2009 Comet assay measurements: a perspective. *Cell Biology and Toxicology* **25** 53–64. (https://doi.org/10.1007/s10565-007-9043-9)

Kuo HW, Yang JS & Chiu MC 2002 Determination of urinary and salivary cotinine using gas and liquid chromatography and enzyme-linked immunosorbent assay. *Journal of Chromatography: B, Analytical Technologies in the Biomedical and Life Sciences* **768** 297–303. (https://doi.org/10.1016/s1570-0232(01)00613-4)

Lavery MR, Acharya P, Sivo SA & Xu L 2019 Number of predictors and multicollinearity: what are their effects on error and bias in regression? *Communications in Statistics: Simulation and Computation* **48** 27–38. (https://doi.org/10.1080/03610918.2017.1371750)

Layali I, Tahmasbpour E, Joulaei M, Jorsaraei SGA & Farzanegi P 2015 Total antioxidant capacity and lipid peroxidation in semen of patient with hyperviscosity. *Cell Journal* **16** 554–559. (https://doi.org/10.22074/cellj.2015.500)

Lee RF & Steinert S 2003 Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. *Mutation Research* **544** 43–64. (https://doi.org/10.1016/s1383-5742(03)00017-6)

Lee SF & Pervaiz S 2011 Assessment of oxidative stress-induced DNA damage by immunoflourescent analysis of 8-oxodG. *Methods in Cell Biology* **103** 99–113. (https://doi.org/10.1016/B978-0-12-385493-3.00005-X)

Lee E, Oh E, Lee J, Sul D & Lee J 2004 Use of the tail moment of the lymphocytes to evaluate DNA damage in human biomonitoring studies. *Toxicological Sciences* **81** 121–132. (https://doi.org/10.1093/toxsci/kfh184)

Lewis SE, John Aitken R, Conner SJ, Iuliis GD, Evenson DP, Henkel R, Giwercman A & Gharagozloo P 2013 The impact of sperm DNA damage in assisted conception and beyond: recent advances in diagnosis and treatment. *Reproductive Biomedicine Online* **27** 325–337. (https://doi.org/10.1016/j.rbmo.2013.06.014)

Lindley EM, Jacobson JD, Corselli J, King A & Chan PJ 2001 Cryopreservation of human cumulus cells for co-cultures and assessment of DNA damage after thawing using the comet assay. *Journal of Assisted Reproduction and Genetics* **18** 534–538. (https://doi.org/10.1023/a:1011991806423)

Linschooten JO, Laubenthal J, Cemeli E, Baumgartner A, Anderson D, Sipinen VE, Brunborg G, Haenen GR, Fthenou E, Briede JJ, et al. 2011 Incomplete protection of genetic integrity of mature spermatozoa against oxidative stress. *Reproductive Toxicology* **32** 106–111. (https://doi.org/10.1016/j.reprotox.2011.05.004)

Liu Y, Yu Z, Zhao S, Cheng L, Man Y, Gao X & Zhao H 2021 Oxidative stress markers in the follicular fluid of patients with polycystic ovary syndrome correlate with a decrease in embryo

quality. *Journal of Assisted Reproduction and Genetics* **38** 471–477. (https://doi.org/10.1007/s10815-020-02014-y)

Mancini A, Festa R, Di Donna V, Leone E, Littarru GP, Silvestrini A, Meucci E & Pontecorvi A 2010 Hormones and antioxidant systems: role of pituitary and pituitary-dependent axes. *Journal of Endocrinological Investigation* **33** 422–433. (https://doi.org/10.1007/BF03346615)

Mancini A, Di Segni C, Raimondo S, Olivieri G, Silvestrini A, Meucci E & Curro D 2016 Thyroid hormones, oxidative stress, and inflammation. *Mediators of Inflammation* **2016** 6757154. (https://doi.org/10.1155/2016/6757154)

Marnett LJ 1999 Lipid peroxidation-DNA damage by malondialdehyde. *Mutation Research* **424** 83–95. (https://doi.org/10.1016/s0027-5107(99)00010-x)

Mecocci P, Polidori MC, Ingegni T, Cherubini A, Chionne F, Cecchetti R & Senin U 1998 Oxidative damage to DNA in lymphocytes from AD patients. *Neurology* **51** 1014–1017. (https://doi.org/10.1212/wnl.51.4.1014)

Micillo A, Vassallo MR, Cordeschi G, D'Andrea S, Necozione S, Francavilla F, Francavilla S & Barbonetti A 2016 Semen leukocytes and oxidative-dependent DNA damage of spermatozoa in male partners of subfertile couples with no symptoms of genital tract infection. *Andrology* **4** 808–815. (https://doi.org/10.1111/andr.12188)

Mintziori G, Kita M, Duntas L & Goulis DG 2016 Consequences of hyperthyroidism in male and female fertility: pathophysiology and current management. *Journal of Endocrinological Investigation* **39** 849–853. (https://doi.org/10.1007/s40618-016-0452-6)

Moller P, Danielsen PH, Karottki DG, Jantzen K, Roursgaard M, Klingberg H, Jensen DM, Christophersen DV, Hemmingsen JG, Cao Y, et al. 2014 Oxidative stress and inflammation generated DNA damage by exposure to air pollution particles. *Mutation Research: Reviews in Mutation Research* **762** 133–166. (https://doi.org/10.1016/j.mrrev.2014.09.001)

Nakamura H, Kimura T, Nakajima A, Shimoya K, Takemura M, Hashimoto K, Isaka S, Azuma C, Koyama M & Murata Y 2002 Detection of oxidative stress in seminal plasma and fractionated sperm from subfertile male patients. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **105** 155–160. (https://doi.org/10.1016/s0301-2115(02)00194-x)

Nicopoullos J, Vicens-Morton A, Lewis SEM, Lee K, Larsen P, Ramsay J, Yap T & Minhas S 2019 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. *Human Reproduction* **34** 1915–1923. (https://doi.org/10.1093/humrep/dez151)

Nunez-Calonge R, Cortes S, Gutierrez Gonzalez LM, Kireev R, Vara E, Ortega L, Caballero P, Rancan L & Tresguerres J 2016 Oxidative stress in follicular fluid of young women with low response compared with fertile oocyte donors. *Reproductive Biomedicine Online* **32** 446–456. (https://doi.org/10.1016/j.rbmo.2015.12.010)

O'Brien RM 2007 A caution regarding rules of thumb for variance inflation factors. *Quality and Quantity* **41** 673–690. (https://doi.org/10.1007/s11135-006-9018-6)

Obuobie K & Jones MK 2003 Hyperthyroidism with low thyroid hormone. *Journal of the Royal Society of Medicine* **96** 185–186. (https://doi.org/10.1258/jrsm.96.4.185)

Oremosu AA & Akang EN 2015 Impact of alcohol on male reproductive hormones, oxidative stress and semen parameters in Sprague–Dawley rats. *Middle East Fertility Society Journal* **20** 114–118. (https://doi.org/10.1016/j.mefs.2014.07.001)

Oyawoye O, Abdel Gadir A, Garner A, Constantinovici N, Perrett C & Hardiman P 2003 Antioxidants and reactive oxygen species in follicular fluid of women undergoing IVF: relationship to outcome. *Human Reproduction* **18** 2270–2274. (https://doi.org/10.1093/humrep/deg450)

Ozkaya MO & Naziroglu M 2010 Multivitamin and mineral supplementation modulates oxidative stress and antioxidant vitamin levels in serum and follicular fluid of women undergoing in vitro fertilization. *Fertility and Sterility* **94** 2465–2466. (https://doi.org/10.1016/j.fertnstert.2010.01.066)

Park JW & Floyd RA 1992 Lipid peroxidation products mediate the formation of 8-hydroxydeoxyguanosine in DNA. *Free Radical Biology and Medicine* **12** 245–250. (https://doi.org/10.1016/0891-5849(92)90111-s)

Pasqualotto FF, Sharma RK, Nelson DR, Thomas AJ & Agarwal A 2000 Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. *Fertility and Sterility* **73** 459–464. (https://doi.org/10.1016/s0015-0282(99)00567-1)

Pasqualotto EB, Agarwal A, Sharma RK, Izzo VM, Pinotti JA, Joshi NJ & Rose BI 2004 Effect of oxidative stress in follicular fluid on the outcome of assisted reproductive procedures. *Fertility and Sterility* **81** 973–976. (https://doi.org/10.1016/j.fertnstert.2003.11.021)

Pasqualotto FF, Umezu FM, Salvador M, Borges Jr E, Sobreiro BP & Pasqualotto EB 2008 Effect of cigarette smoking on antioxidant levels and presence of leukocytospermia in infertile men: a prospective study. *Fertility and Sterility* **90** 278–283. (https://doi.org/10.1016/j.fertnstert.2008.02.123)

Pasqualotto EB, Lara LV, Salvador M, Sobreiro BP, Borges E & Pasqualotto FF 2009 The role of enzymatic antioxidants detected in the follicular fluid and semen of infertile couples undergoing assisted reproduction. *Human Fertility* **12** 166–171. (https://doi.org/10.1080/14647270903207941)

Paszkowski T 1998 Concentration gradient of cotinine between blood serum and preovulatory follicular fluid. *Ginekologia Polska* **69** 1131–1136.

Peñarrubia J, Fábregues F, Creus M, Manau D, Casamitjana R, Guimerá M, Carmona F, Vanrell JA & Balasch J 2003 LH serum levels during ovarian stimulation as predictors of ovarian response and assisted reproduction outcome in down-regulated women stimulated with recombinant FSH. *Human Reproduction* **18** 2689–2697. (https://doi.org/10.1093/humrep/deg506)

Pilger A & Rüdiger HW 2006 8-Hydroxy-2′-deoxyguanosine as a marker of oxidative DNA damage related to occupational and environmental exposures. *International Archives of Occupational and Environmental Health* **80** 1–15. (https://doi.org/10.1007/s00420-006-0106-7)

Raghunath A, Sundarraj K, Nagarajan R, Arfuso F, Bian J, Kumar AP, Sethi G & Perumal E 2018 Antioxidant response elements: discovery, classes, regulation and potential applications. *Redox Biology* **17** 297–314. (https://doi.org/10.1016/j.redox.2018.05.002)

Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S & Dhama K 2014 Oxidative stress, prooxidants, and antioxidants: the interplay. *BioMed Research International* **2014** 761264. (https://doi.org/10.1155/2014/761264)

Rakhit M, Gokul SR, Agarwal A & Du Plessis SS 2013 Antioxidant strategies to overcome OS in IVF-embryo transfer. In *Studies on Women's Health*. Eds **A Agarwal, N Aziz & B Rizk.** Totowa, NJ: Humana Press.

Raman RS, Chan PJ, Corselli JU, Patton WC, Jacobson JD, Chan SR & King A 2001 Comet assay of cumulus cell DNA status and the relationship to oocyte fertilization via intracytoplasmic sperm injection. *Human Reproduction* **16** 831–835. (https://doi.org/10.1093/humrep/16.5.831)

Ramzan MH, Ramzan M, Khan MM, Ramzan F, Wahab F, Khan MA, Jillani M & Shah M 2015 Human semen quality and sperm DNA damage assessed by comet assay in clinical groups. *Turkish Journal of Medical Sciences* **45** 729–737. (https://doi.org/10.3906/sag-1407-50)

Revelli A, Canosa S, Bergandi L, Skorokhod OA, Biasoni V, Carosso A, Bertagna A, Maule M, Aldieri E, D'Eufemia MD, et al. 2017 Oocyte polarized light microscopy, assay of specific follicular fluid metabolites, and gene expression in cumulus cells as different approaches to predict fertilization efficiency after ICSI. *Reproductive Biology and Endocrinology* **15** 47. (https://doi.org/10.1186/s12958-017-0265-2)

Rosen EM, Mínguez-Alarcón L, Meeker JD, Williams PL, Milne GL, Hauser R, Ferguson KK & EARTH Study Team 2019 Urinary oxidative stress biomarker levels and reproductive outcomes among couples undergoing fertility treatments. *Human Reproduction* **34** 2399–2409. (https://doi.org/10.1093/humrep/dez228)

Roychoudhury S, Sharma R, Sikka S & Agarwal A 2016 Diagnostic application of total antioxidant capacity in seminal plasma to assess oxidative stress in male factor infertility. *Journal of Assisted Reproduction and Genetics* **33** 627–635. (https://doi.org/10.1007/s10815-016-0677-5)

Ruder EH, Hartman TJ, Blumberg J & Goldman MB 2008 Oxidative stress and antioxidants: exposure and impact on female fertility. *Human Reproduction Update* **14** 345–357. (https://doi.org/10.1093/humupd/dmn011)

Sabeti P, Pourmasumi S, Rahiminia T, Akyash F & Talebi AR 2016 Etiologies of sperm oxidative stress. *International Journal of Reproductive Biomedicine* **14** 231–240. (https://doi.org/10.29252/ijrm.14.4.231)

Sakamoto Y, Ishikawa T, Kondo Y, Yamaguchi K & Fujisawa M 2008 The assessment of oxidative stress in infertile patients with varicocele. *BJU International* **101** 1547–1552. (https://doi.org/10.1111/j.1464-410X.2008.07517.x)

Saleh RA, Agarwal A, Sharma RK, Nelson DR & Thomas Jr AJ 2002 Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. *Fertility and Sterility* **78** 491–499. (https://doi.org/10.1016/s0015-0282(02)03294-6)

Sanocka D & Kurpisz M 2004 Reactive oxygen species and sperm cells. *Reproductive Biology and Endocrinology* **2** 12–12. (https://doi.org/10.1186/1477-7827-2-12)

Schulte RT, Ohl DA, Sigman M & Smith GD 2010 Sperm DNA damage in male infertility: etiologies, assays, and outcomes. *Journal of Assisted Reproduction and Genetics* **27** 3–12. (https://doi.org/10.1007/s10815-009-9359-x)

Seino T, Saito H, Kaneko T, Takahashi T, Kawachiya S & Kurachi H 2002 Eight-hydroxy-2′-deoxyguanosine in granulosa cells is correlated with the quality of oocytes and embryos in an in vitro fertilization-embryo transfer program. *Fertility and Sterility* **77** 1184–1190. (https://doi.org/10.1016/s0015-0282(02)03103-5)

Sharma RK & Agarwal A 1996 Role of reactive oxygen species in male infertility. *Urology* **48** 835–850. (https://doi.org/10.1016/s0090-4295(96)00313-5)

Sharma P, Jha AB, Dubey RS & Pessarakli M 2012 Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany* **2012** 1–26. (https://doi.org/10.1155/2012/217037)

Sharma R, Roychoudhury S, Singh N & Sarda Y 2017 Methods to measure reactive oxygen species (ROS) and total antioxidant capacity (TAC) in the reproductive system. In *Oxidative Stress in Human Reproduction: Shedding Light on a Complicated Phenomenon*. Eds **A Agarwal, R Sharma, S Gupta, A Harlev, G Ahmad, SS Du Plessis, SC Esteves, SM Wang & D Durairajanayagam.** Cham: Springer International Publishing.

Shen H & Ong C 2000 Detection of oxidative DNA damage in human sperm and its association with sperm function and male infertility. *Free Radical Biology and Medicine* **28** 529–536. (https://doi.org/10.1016/s0891-5849(99)00234-8)

Shi X, Chan DYL, Zhao MP, Chan CPS, Huang J & Li TC 2019 Comparison of DNA fragmentation levels in spermatozoa with different sex chromosome complements. *Reproductive Biomedicine Online* **38** 56–65. (https://doi.org/10.1016/j.rbmo.2018.10.005)

Simon L & Carrell DT 2013 Sperm DNA damage measured by comet assay. *Methods in Molecular Biology* **927** 137–146. (https://doi.org/10.1007/978-1-62703-038-0_13)

Simon L, Emery BR & Carrell DT 2017 Review: diagnosis and impact of sperm DNA alterations in assisted reproduction. *Best Practice and Research: Clinical Obstetrics and Gynaecology* **44** 38–56. (https://doi.org/10.1016/j.bpobgyn.2017.07.003)

Singh NP, Mccoy MT, Tice RR & Schneider EL 1988 A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research* **175** 184–191. (https://doi.org/10.1016/0014-4827(88)90265-0)

Singh NP, Muller CH & Berger RE 2003 Effects of age on DNA double-strand breaks and apoptosis in human sperm. *Fertility and Sterility* **80** 1420–1430. (https://doi.org/10.1016/j.fertnstert.2003.04.002)

Singh AK, Chattopadhyay R, Chakravarty B & Chaudhury K 2013 Markers of oxidative stress in follicular fluid of women with endometriosis and tubal infertility undergoing IVF. *Reproductive Toxicology* **42** 116–124. (https://doi.org/10.1016/j.reprotox.2013.08.005)

Sinko I, Morocz M, Zadori J, Kokavszky K & Rasko I 2005 Effect of cigarette smoking on DNA damage of human cumulus cells analyzed by comet assay. *Reproductive Toxicology* **20** 65–71. (https://doi.org/10.1016/j.reprotox.2004.12.007)

Tamura H, Takasaki A, Miwa I, Taniguchi K, Maekawa R, Asada H, Taketani T, Matsuoka A, Yamagata Y, Shimamura K, et al. 2008 Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. *Journal of Pineal Research* **44** 280–287. (https://doi.org/10.1111/j.1600-079X.2007.00524.x)

Tamura H, Takasaki A, Nakamura Y, Numa F & Sugino N 2014 A pilot study to search possible mechanisms of ultralong gonadotropin-releasing hormone agonist therapy in IVF-ET patients with endometriosis. *Journal of Ovarian Research* **7** 100. (https://doi.org/10.1186/s13048-014-0100-8)

Tang LX, Yuan DJ, Wang QL, Jiang F, Guo J, Tang YG, Zheng LX & Kang JX 2016 Association of decreased spermatozoa omega-3 fatty acid levels and increased oxidative DNA damage with varicocele in infertile men: a case control study. *Reproduction, Fertility, and Development* **28** 648–654. (https://doi.org/10.1071/RD14276)

Tarlatzis BC, Zepiridis L, Grimbizis G & Bontis J 2003 Clinical management of low ovarian response to stimulation for IVF: a systematic review. *Human Reproduction Update* **9** 61–76. (https://doi.org/10.1093/humupd/dmg007)

Tatemoto H, Sakurai N & Muto N 2000 Protection of porcine oocytes against apoptotic cell death caused by oxidative stress during in vitro maturation: role of cumulus cells. *Biology of Reproduction* **63** 805–810. (https://doi.org/10.1095/biolreprod63.3.805)

Tchou J & Grollman AP 1993 Repair of DNA containing the oxidatively-damaged base, 8-oxoguanine. *Mutation Research* **299** 277–287. (https://doi.org/10.1016/0165-1218(93)90104-l)

Terasaka T, Adakama ME, Li S, Kim T, Terasaka E, Li D & Lawson MA 2017 Reactive oxygen species link Ggonadotropin-releasing hormone receptor signaling cascades in the gonadotrope. *Frontiers in Endocrinology* **8** 286. (https://doi.org/10.3389/fendo.2017.00286)

Terzioğlu F, Karahalil B, Yücel Ç & Türk R 2016 The effect of cotinine concentrations in seminal plasma and follicular fluid on the pregnancy outcomes of couples undergoing assisted reproductive techniques. *Turkish Journal of Medical Sciences* **46** 1495–1501. (https://doi.org/10.3906/sag-1504-121)

Thaker R, Mishra V, Gor M, Agarwal R, Sheth H, Kapadia P & Kumar S 2020 The role of stimulation protocol, number of oocytes

retrieved with respect to follicular fluid oxidative stress and IVF outcome. *Human Fertility* **23** 23–31. (https://doi.org/10.1080/14647273.2018.1551630)

Tola EN, Koşar PA, Karatopuk DU, Sancer O & Oral B 2019 Effect of DNA damage of cumulus oophorus cells and lymphocytes analyzed by alkaline comet assay on oocyte quality and intracytoplasmic sperm injection success among patients with polycystic ovary syndrome. *Journal of Obstetrics and Gynaecology Research* **45** 609–618. (https://doi.org/10.1111/jog.13868)

Ulloa-Aguirre A & Lira-Albarran S 2016 Clinical applications of gonadotropins in the male. *Progress in Molecular Biology and Translational Science* **143** 121–174. (https://doi.org/10.1016/bs.pmbts.2016.08.003)

Uppangala S, Fernandes G, Salian SR, Kumar P, Talevi R, Kalthur G & Adiga SK 2020 Reduced ovarian response to controlled ovarian stimulation is associated with increased oxidative stress in the follicular environment. *Reproductive Biology* **20** 402–407. (https://doi.org/10.1016/j.repbio.2020.04.005)

Vannuccini S, Clifton VL, Fraser IS, Taylor HS, Critchley H, Giudice LC & Petraglia F 2016 Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome. *Human Reproduction Update* **22** 104–115. (https://doi.org/10.1093/humupd/dmv044)

Varnagy A, Koszegi T, Gyorgyi E, Szegedi S, Sulyok E & Premusz V 2018 Levels of total antioxidant capacity and 8-hydroxy-2′-deoxyguanosine of serum and follicular fluid in women undergoing in vitro fertilization: focusing on endometriosis. *Human Fertility* 1–9.

Várnagy Á, Kőszegi T, Györgyi E, Szegedi S, Sulyok E, Prémusz V & Bódis J 2020 Levels of total antioxidant capacity and 8-hydroxy-2′-deoxyguanosine of serum and follicular fluid in women undergoing in vitro fertilization: focusing on endometriosis. *Human Fertility* **23** 200–208. (https://doi.org/10.1080/14647273.2018.1535719)

Vatannejad A, Tavilani H, Sadeghi MR, Amanpour S, Shapourizadeh S & Doosti M 2017 Evaluation of ROS-TAC score and DNA damage in fertile normozoospermic and infertile asthenozoospermic males. *Urology Journal* **14** 2973–2978.

Venkatesh S, Shamsi MB, Dudeja S, Kumar R & Dada R 2011 Reactive oxygen species measurement in neat and washed semen: comparative analysis and its significance in male infertility assessment. *Archives of Gynecology and Obstetrics* **283** 121–126. (https://doi.org/10.1007/s00404-010-1645-4)

Villanueva I, Alva-Sánchez C & Pacheco-Rosado J 2013 The role of thyroid hormones as inductors of oxidative stress and neurodegeneration. *Oxidative Medicine and Cellular Longevity* **2013** 218145.

Vine MF, Hulka BS, Margolin BH, Truong YK, Hu PC, Schramm MM, Griffith JD, Mccann M & Everson RB 1993 Cotinine concentrations in semen, urine, and blood of smokers and nonsmokers. *American Journal of Public Health* **83** 1335–1338. (https://doi.org/10.2105/ajph.83.9.1335)

Wdowiak A & Wdowiak A 2015 Comparing antioxidant enzyme levels in follicular fluid in ICSI-treated patients. *Gynecologie, Obstetrique and Fertilite* **43** 515–521. (https://doi.org/10.1016/j.gyobfe.2015.06.004)

Zandieh Z, Vatannejad A, Doosti M, Zabihzadeh S, Haddadi M, Bajelan L, Rashidi B & Amanpour S 2018 Comparing reactive oxygen species and DNA fragmentation in semen samples of unexplained infertile and healthy fertile men. *Irish Journal of Medical Science* **187** 657–662. (https://doi.org/10.1007/s11845-017-1708-7)

Zenzes MT, Puy LA & Bielecki R 1997 Immunodetection of cotinine protein in granulosa-lutein cells of women exposed to cigarette smoke. *Fertility and Sterility* **68** 76–82. (https://doi.org/10.1016/s0015-0282(97)81479-3)

Zhuo L & Kimata K 2001 Cumulus oophorus extracellular matrix: its construction and regulation. *Cell Structure and Function* **26** 189–196. (https://doi.org/10.1247/csf.26.189)

Zubair M, Ahmad M & Qureshi ZI 2017 Review on arsenic-induced toxicity in male reproductive system and its amelioration. *Andrologia* **49**. (https://doi.org/10.1111/and.12791)

Received in final form 15 April 2021
Accepted 23 April 2021
Accepted Manuscript published online 23 April 2021