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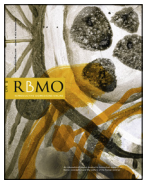

ARTICLE

Predictive value of seminal oxidation-reduction potential analysis for reproductive outcomes of ICSI

BIOGRAPHY

Ralf Henkel studied Biology/Chemistry in Marburg, Germany, and obtained his PhD in 1990. In 2004, he became Professor in the Department of Urology at the University of Jena, and at UWC, Bellville, South Africa, from 2005 until 2020. Since 2020, he has been at LogixX Pharma and Imperial College London, UK.

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KEY MESSAGE

Seminal oxidation-reduction potential (ORP) is predictive of fertilization, blastocyst development, implantation, clinical pregnancy and live birth after ICSI. Because ORP measurement showed comparable predictive value to the TUNEL assay, it can be a quicker and cheaper alternative to the determination of DNA damage.

ABSTRACT

Research question: Is seminal oxidation-reduction potential (ORP) clinically relevant to reproductive outcome?

Design: Prospective observational study including a total of 144 couples who had an intracytoplasmic sperm injection (ICSI) cycle between June 2018 and December 2020. The study included patients undergoing fresh ICSI cycles with autologous gametes. Cycles that had day 3 embryo transfers and cryopreservation cycles were excluded. There was no restriction on patients with severe male infertility; couples with unexplained infertility and unexplained male infertility were included, those with azoospermia were excluded. Semen analysis, seminal ORP as determined by means of the MiOXSYS system, sperm DNA fragmentation (SDF) and reproductive outcomes (fertilization, blastocyst development, clinical pregnancy and live birth) were determined.

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KEYWORDS

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Results: Seminal ORP was significantly negatively correlated with fertilization rate (r = –0.267; P = 0.0012), blastocyst development rate (r = –0.432; P < 0.0001), implantation/clinical pregnancy (r = –0.305; P = 0.0003) and live birth (r = –0.366; P < 0.0001). Receiver operating characteristic curve analysis showed significant predictive power for ORP for fertilization (≥80%; area under the curve [AUC] 0.652; P = 0.0012), blastocyst development rate (≥60%; AUC 0.794; P < 0.0001), implantation/clinical pregnancy (AUC 0.680; P = 0.0002) and live birth (AUC 0.728; P < 0.0001). Comparable results were obtained for SDF (fertilization: AUC 0.678; blastocyst development: AUC 0.777; implantation/clinical pregnancy: AUC 0.665; live birth: AUC 0.723). Normal sperm morphology showed the lowest predictive power for all reproductive outcome parameters. With male age as confounding factor, ORP (cut-off value of 0.51 mV/10^6 sperm/ml) has significant (P < 0.04667) effects on odds ratios for all reproductive outcome parameters. Multivariate logistic regression to investigate potential seminal and female confounding factors revealed that seminal ORP significantly (P < 0.0039; P < 0.0130) affects reproductive outcome.

Conclusion: Seminal ORP is relevant for good fertilization, blastocyst development, implantation, clinical pregnancy and live birth.

INTRODUCTION

Globally, nearly 70 million people are affected by infertility. According to the World Health Organization, an estimated 9% of couples worldwide are unable to conceive and up to 50% of infertility cases are attributable to male factors (*Boivin et al., 2007*). Despite advances in understanding the causes of male infertility, idiopathic infertility still accounts for about 30–50% of male infertility issues (*Gelbaya et al., 2014*). Many causes of male infertility have been identified and can be classified into pre-testicular, testicular or post-testicular with congenital, acquired or idiopathic factors (*Agarwal et al., 2020*, *2021a*). In many of the underlying factors such as lifestyle choices, infections, varicocele, radiation or environmental pollution, oxidative stress is involved, eventually causing sperm DNA fragmentation (SDF) (*Agarwal et al., 2020*).

Oxidative stress has been reported to play an important role in various aetiologies of male infertility including male genital tract infection/inflammation, varicocele or adverse lifestyle conditions and related diseases such as obesity or diabetes mellitus (*Abbasihormozi et al., 2019*; *Agarwal et al., 2006*; *Aitken et al., 2014*; *Wright et al., 2014*). Oxidative stress is induced by the imbalance between reactive oxygen species (ROS) and antioxidants. Under normal conditions, ROS and antioxidants are in balance; antioxidants neutralize excessive amounts of these highly reactive free radicals and maintain homeostasis (*Kothari et al., 2010*). Under pathological conditions, when there are higher concentrations of ROS than antioxidants, ROS-mediated oxidative stress ensues. Because sperm plasma membranes have an extraordinarily high content of polyunsaturated fatty acids (*Henkel, 2011*; *Parks and Lynch, 1992*), male germ cells are highly susceptible to lipid peroxidation (*Gharagozloo and Aitken, 2011*). Oxidative stress is also regarded as a major cause of SDF (*Aitken and Clarkson, 1987*; *Muratori et al., 2015a*). There is a growing body of evidence indicating important roles for seminal oxidative stress and SDF for male infertility (*Esteves et al., 2021*) and the reproductive outcomes of assisted reproduction (*Agarwal et al., 2021b*; *Esteves et al., 2015*; *Sakkas and Alvarez, 2010*). In a systematic review and meta-analysis including 23 studies with a total of 6771 cycles of treatment with assisted reproductive technology (ART), *Deng et al. (2019)* reported that high SDF was associated with a significantly increased risk of poorer cycle outcome. In another systematic review and meta-analysis of 27 studies with 1941 patients with unexplained recurrent spontaneous abortion, *Yifu et al. (2020)* reported a significant association between SDF and recurrent pregnancy loss.

In order to predict male fertility and sperm fertilizing capacity, especially for assisted reproduction procedures, various semen parameters and sperm functions including normal sperm morphology (*Kruger et al., 1988*; *Obara et al., 2001*) or SDF (*Simon et al., 2017*) are used and have proved to be valuable tools in the clinical work-up of male infertility. For seminal oxidative stress and ROS assessment in particular, luminometric techniques with luminol or lucigenin as chemiluminescent probes are usually used (*Aitken et al., 1992*; *Zorn et al., 2003*). However, despite the simplicity of the luminometric measurement, the direct determination of ROS in semen is technically challenging, and assays are therefore not validated. For the determination of SDF, several methods with different protocols have been described. The most frequently used techniques to determine SDF and thereby predict the probability of a successful outcome of treatment with ART are the terminal deoxynucleotidyl transferase-mediated dUDP nick-end labelling (TUNEL) assay (*Henkel et al., 2004*; *Sharma et al., 2010*), the sperm chromatin structure assay (SCSA; *Jerre et al., 2019*), the comet assay (*Simon et al., 2011*) and the sperm chromatin dispersion (SCD) assay (*Muriel et al., 2006*). On the other hand, the different methods described for SDF actually measure different aspects of sperm DNA damage (*Henkel et al., 2010*; *Zini and Sigman, 2009*), resulting in only moderate correlation between the different methods (*Rex et al., 2017*). In addition, extensive preparation (TUNEL assay), labour-intensive procedure (comet assay), small number of evaluated cells (SCD, comet assay) and poor predictive accuracy for IVF and intracytoplasmic sperm insemination (ICSI) outcome (SCSA) result in a lack of strong evidence (*Cissen et al., 2016*; *Rex et al., 2017*; *Simon et al., 2019*) and therefore lack of agreement over using these tests.

A newly introduced method of detecting seminal oxidative stress is to measure the oxidation-reduction potential (ORP), which provides the overall balance between oxidants and antioxidants (*Agarwal et al., 2016*). This method has been shown to be a quick, easy, reliable and cost-effective tool to identify infertile men (*Agarwal et al., 2018*). In addition, *Arafa et al. (2019)* and *Elbardisi et al. (2020)* have shown a mediocre but highly significant positive association between SDF and seminal ORP. Because of the reported issues including labour-

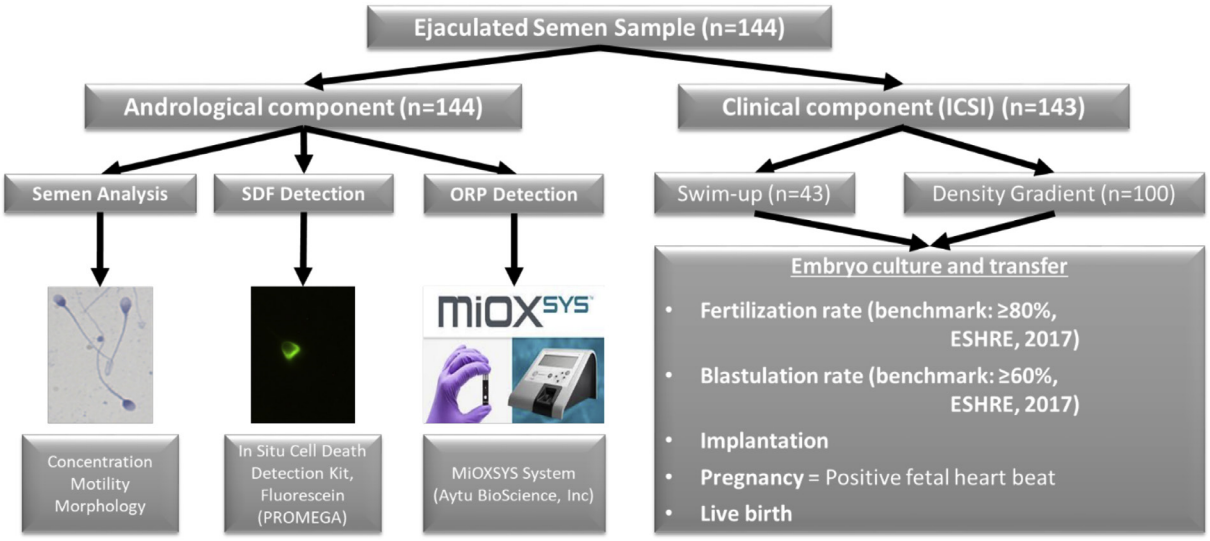

FIGURE 1 Study design and project methodology. ICSI = intracytoplasmic sperm injection; ORP = oxidation-reduction potential; SDF = sperm DNA fragmentation.

intensive and costly procedures with the different techniques to determine SDF, there is still a need for diagnostic tools to improve the prediction of male fertility potential. In contrast to methods for SDF determination, measurement of ORP is easy and quick, but has not yet been evaluated to predict male fertility potential in an assisted reproduction set-up. Therefore, the aim of this prospective study was to investigate the predictive capabilities of seminal ORP for reproductive outcomes (fertilization, blastocyst development, clinical pregnancy and live birth) in patients undergoing ICSI cycles and compare these with the predictive potential of a standard fluorescent TUNEL assay for SDF.

MATERIALS AND METHODS

Setting and subjects

The current prospective observational study was approved on 13 January 2017 by the Biomedical Ethics Committee (reference number: BM/16/5/18) of the University of the Western Cape, Bellville, South Africa, and all participating clinics. The study was conducted from June 2018 to December 2020. A total of 144 patients (one cycle each that was included in the study) undergoing ICSI treatments at Drs Aevitas Fertility Clinic, Vincent Palotti Hospital, Pinelands, South Africa; Cape Fertility Clinic, Claremont, South Africa; and Tygerberg Fertility Clinic, Institute for Reproductive Medicine, Tygerberg, South Africa, were enrolled in this study and gave informed consent. The study included consenting patients undergoing fresh ICSI cycles with autologous gametes. Cycles that had day 3 embryo transfers and cryopreservation cycles were excluded. There was no restriction on the inclusion of patients with severe male factor infertility; couples with unexplained infertility and unexplained male infertility were included, while those with azoospermia were excluded. On the female side, patients with primary and secondary infertility, unexplained infertility, polycystic ovaries, endometriosis, tubal factor, myomectomy, as well as ovarian insufficiency and advanced maternal age, were included.

Study procedures

Patients were instructed to abstain from sexual intercourse 2 to 3 days prior to providing the semen sample. The ejaculated semen samples were produced by masturbation, collected in a sterile specimen container and kept at 37°C until liquefaction was observed. Andrological diagnostics (i.e. manual semen analysis, testing for SDF and determination of seminal ORP) were conducted in 180 µl (50 µl for standard semen analysis; 30 µl for ORP; 100 µl for SDF) of the semen samples that were provided for the ICSI procedure within 30 min of liquefaction. The rest of the sample was either subjected to density gradient centrifugation or swim-up based on total motile count before the ICSI procedure (FIGURE 1).

ART procedure

Stimulation protocol

For ovarian stimulation, the flexible antagonist protocol was used, consisting of daily gonadotrophins (300 IU FSH in a step-down fashion to 150 IU) for 5 days beginning on day 3 of the menstrual cycle. Administration of gonadotrophin-releasing hormone (GnRH) antagonist (0.25 mg) was started by s.c. injection when the leading follicle measured 14 mm or more. Ovulation was induced by s.c. or i.m. administration of 10,000 IU human chorionic gonadotrophin (HCG) or 200 µg GnRH agonist when the lead follicle was ≥18 mm and at least two other follicles were ≥16 mm in size. Oocyte retrieval was performed within 34–36 h after HCG administration. Luteal phase support was achieved by vaginal progesterone preparation if HCG was used.

Semen processing for ICSI

For sperm processing, standardized methods conforming to *World Health Organization (2010)* were followed across the participating clinics. The preferred method for sperm processing was determined by the initial evaluation of semen parameters and quality of the semen sample produced. Two basic methods, swim-up and density gradient centrifugation (Sil-Select; FertiPro, Harrilabs, Cape Town, South Africa), were used. For the swim-up, ORIGIO Sperm Wash medium was used, while Sperm Preparation Medium (CooperSurgical, Ferring,

Cape Town, South Africa) was used for wash procedures and Sil-Silect Plus™ (FertiPro; 40%/80%) for the density gradient centrifugation.

Oocyte retrieval procedure

Oocytes were retrieved under conscious sedation (i.v. Dormicum®, Hoffmann-La Roche Ltd, Basel, Switzerland) using transvaginal ultrasound guidance. Follicular fluid was aspirated using sonar-guided ultrasound, and examined for the presence of cumulus–oocyte complexes (COC) both macroscopically and microscopically. The oocytes within the COC were superficially graded to infer maturity (immature, germinal vesicles [GV] or metaphase I [MI] oocytes; mature, metaphase II [MII]). Post-grading, the COC were collected, washed in Quinn's Advantage™ Medium with HEPES (SAGE™), and transferred to a Fert™ (ORIGIO®) medium.

ICSI and embryo culture

All embryology procedures were performed by a fully trained and experienced embryologist. For this study, all included oocytes were injected by standard ICSI. For all preparatory and culture procedures, Quinn's Advantage™ (Harrilabs) sequential culture medium range was used including HEPES-buffered medium, sperm preparation medium, fertilization/cleavage and blastocyst medium, oil for tissue culture, hyaluronidase and polyvinylpyrrolidone. A standardized, routine method was used for all patients. After incubation for 16–18 h, oocytes were assessed for fertilization (visualization of two pronuclei [2PN]). Embryos were individually cultured and evaluated for embryo quality (blastomere morphology, size and percentage of fragmentation) on day 2 and day 3 post-insemination. Culture to the blastocyst stage (day 4 to day 6 post-insemination) was performed in SAGE one-step culture medium (CooperSurgical). Embryo culture was performed in Planer BT37 incubators (Planer, Sunbury-on-Thames, UK) under 5% O_2 and 7.2% CO_2.

Embryo transfer and reproductive outcome parameters

Embryo transfers were performed using Wallace® Classic Embryo Transfer Catheters (CooperSurgical) under standard ultrasound guidance (*Sallam and Sadek, 2003*) on day 5. According to embryo transfer guidelines published by the Southern African Society for Reproductive Medicine and Gynaecological Endoscopy (SASREG), patient history and conforming to national legislation requirements, no more than three embryos were transferred by a reproductive medicine specialist and clinical embryologist. Embryos were assessed and graded on day 5 (blastocyst stage). Embryo grading was performed under high magnification according to a modified grading system (*Richardson et al., 2015*).

As study outcome parameters, fertilization (number of oocytes with 2PN stage/total MII oocytes) and blastocyst formation rates (developed blastocysts per fertilized oocyte), as well as the clinical parameters implantation (proportion of embryo transfers with at least one gestational sac visualized on ultrasound per total number of embryo transfers), clinical pregnancy (as determined by fetal heartbeat), miscarriage (premature loss of the fetus up to 23 weeks) and live birth (delivery of at least one live baby) rates were recorded. For the classification of good and poor fertilization and blastocyst development, the benchmark values for the key performance indicators (fertilization: ≥80%; blastocyst development: ≥60%) according to the Vienna consensus (*ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine, 2017*) were used. For implantation, clinical pregnancy and live birth, classification for receiver operating characteristic (ROC) curve analyses was performed on a yes/no basis. Clinical pregnancy and live birth were analysed per embryo transfer.

Andrology laboratory procedure

Semen analysis

All semen samples were assessed for sperm concentration and motility according to *World Health Organization (2010)* criteria with standard quality control measures being applied. For the determination of normal sperm morphology, a smear of 10 µl semen was applied on a slide, stained according to the Papanicolaou staining protocol and evaluated according to strict criteria (*Menkveld et al., 1990*).

Oxidative stress

Seminal oxidative stress was determined by means of measurement of the ORP using the MiOXSYS system (Aytu Bioscience, Englewood, CO, USA). In brief, 30 µl of liquefied semen were placed on the sample port of the ORP sensor. After about 3–4 min, the result could be read in millivolts (mV) at the display, then normalized according to the sperm concentration and expressed as mV/10^6 sperm/ml (*Agarwal et al., 2019*).

SDF

SDF was measured using the fluorometric TUNEL assay (Promega Corporation, Madison, USA) according to the protocol described previously (*Henkel et al., 2004*). A total of 300 spermatozoa were evaluated for TUNEL positivity under an epifluorescence microscope (Zeiss, Oberkochen, Germany) at 1000 × magnification. Then, the percentage of green fluorescing spermatozoa (TUNEL-positive) was calculated and recorded. Positive and negative controls were run in parallel with the samples.

Statistical analysis

MedCalc® Statistical Software version 20.009 (MedCalc Software Ltd, Ostend, Belgium) was used for the statistical analysis. After testing for normal distribution using the chi-squared test, non-parametric tests were employed for the statistical evaluation. Spearman's rank correlation coefficient was used to determine the numerical correlations for analysed parameters. To determine the predictive capabilities of ORP, SDF and normal sperm morphology for fertilization, blastocyst development rates, clinical pregnancy and live birth, ROC curve analyses were generated according to the DeLong method (*DeLong et al., 1988*).

In order to investigate whether seminal ORP was independently influencing the reproductive outcome, the female variables (age, primary and secondary infertility, unexplained infertility, polycystic ovaries, endometriosis, tubal factor, myomectomy, ovarian insufficiency and advanced maternal age) were included in a stepwise logistic regression model. In addition, a stepwise logistic regression model was used to determine the influence of semen factors (sperm concentration, motility, normal sperm morphology and seminal ORP) on fertilization, blastocyst development, implantation, clinical pregnancy and live birth. Considering that the data for cycles with implantation and clinical pregnancy are the same, these were reported together as 'implantation/clinical pregnancy'.

TABLE 1 SUMMARY STATISTICS OF THE MAIN PARAMETERS ANALYSED IN THIS STUDY

Parameter	No. of cycles	Rate (%)	Minimum	Maximum	Median	Mean ± SD
Female age (years)	144		27.0	46.0	35.0	34.8 ± 3.9
Male age (years)	144		28.0	51.0	37.0	36.5 ± 4.3
Sperm concentration (10^6/ml)	144		2.0	198.0	50.0	53.5 ± 35.9
Motility (%)	144		5.0	88.0	53.0	51.9 ± 19.0
Normal sperm morphology (%)	144		0.0	23.0	4.0	5.3 ± 4.1
ORP (mV/10^6 sperm/ml)	144		0.02	25.3	0.5	0.9 ± 2.2
TUNEL-positive spermatozoa (%)	144		4.0	62.0	22.5	24.3 ± 13.4
Fertilization rate (%; per oocyte injected)	143	96.5	0.0	100.0	80.0	73.5 ± 22.2
Blastocyst development rate (%; per fertilized oocyte)	138	97.1	0.0	100.0	71.4	67.9 ± 24.1
Implantation (rate per transfer)	134	40.3	0.0	100.0	0.0	40.3 ± 49.2
Clinical pregnancy (rate per transfer)	134	40.3	0.0	100.0	0.0	40.3 ± 49.2
Live birth (rate per transfer)	134	29.1	0.0	100.0	0.0	29.1 ± 45.6

Implantation = cycles with ≥1 gestational sac, clinical pregnancy = cycles with ≥ foetal heart beat and live birth = cycles with ≥1 live baby delivered.

ORP = oxidation-reduction potential; TUNEL = terminal deoxynucleotidyl transferase-mediated dUDP nick-end labelling.

As a sub-analysis, men were categorized according to their age as <37 years or ≥37 years by using the median of the male age. The Cochran–Mantel–Haenszel test was used to calculate odds ratios for male age. A *P*-value of <0.05 was considered significant.

RESULTS

A total of 144 patients were recruited for the study, out of whom 143 underwent ICSI treatment and were included in the analysis; in one case no oocytes were obtained. In addition, cryopreservation cycles and day 3 embryo transfers were excluded. The patient cohort comprised 51 couples with primary infertility and 93 with secondary infertility. In detail, 45 patients were recorded with unexplained infertility, 43 women with polycystic ovary syndrome, 31 with endometriosis, 25 with tubal factor, 3 after myomectomy, 14 with ovarian insufficiency, 21 poor responders and 73 women of advanced maternal age (>35 years). Ninety-three patients presented with multiple of these conditions. The summary statistics of patient age, semen parameters (sperm concentration, total motility and normal sperm morphology), SDF and ORP along with fertilization and blastocyst development rates are presented in TABLE 1. Overall, 134 women out of 143 (93.7%) underwent embryo transfer with mean ± SD: 1.8 ± 0.5 embryos per transfer and implantation was observed in 54 patients (40.3%). The embryo transfers resulted in 54 (40.3%) clinical pregnancies and 39 (29.1%) live births, while 15 (11.2%) miscarriages occurred. In 5 out of 143 (3.5%) cases, no fertilization occurred and in 4 out of 138 (2.9%) cases with fertilized oocytes no blastocysts were obtained.

TABLE 2 compares the mean values of seminal ORP and SDF in the cases where no fertilization, blastocyst formation, implantation/clinical pregnancy or live birth, respectively, occurred with those from patients with fertilization, blastocyst formation, implantation, clinical pregnancy and live birth. All comparisons except for ORP (fertilization: $P = 0.6924$ and blastocyst development: $P = 0.0964$) and SDF (fertilization: $P = 0.0914$ and

TABLE 2 COMPARISON OF ORP AND SDF IN CASES WITH OR WITHOUT FERTILIZATION, BLASTOCYST FORMATION, IMPLANTATION, CLINICAL PREGNANCY OR LIVE BIRTH

Parameter	ORP (mV/10^6 sperm/ml)	*P*-value	SDF (%)	*P*-value
Fertilization rate (0%) (n = 5)	0.83 ± 0.82	0.6924	34.20 ± 14.38	0.0914
Fertilization rate (>0%) (n = 138)	0.98 ± 2.22		23.91 ± 13.34	
Blastocyst formation rate (0%) (n = 4)	2.66 ± 2.23	0.0964	31.25 ± 13.84	0.2109
Blastocyst formation rate (>0%) (n = 134)	0.93 ± 2.21		23.47 ± 13.19	
Implantation / clinical pregnancy (no) (n = 80)	0.87 ± 0.67	0.0004	26.56 ± 13.89	0.0012
Implantation / clinical pregnancy (yes) (n = 54)	1.00 ± 3.40		18.88 ± 10.61	
Live birth (no) (n = 95)	0.86 ± 0.65	<0.0001	26.53 ± 13.63	0.0001
Live birth (yes) (n = 39)	1.09 ± 4.00		16.63 ± 9.53	

Values reported as mean ± SD.

Implantation = cycles with ≥1 gestational sac, clinical pregnancy = cycles with ≥ foetal heart beat and live birth = cycles with ≥1 live baby delivered.

The significance was calculated using the Mann–Whitney test.

P-values <0.05 indicate statistical significance.

ORP = oxidation-reduction potential; SDF = sperm DNA fragmentation.

TABLE 3 COMPARISON OF ORP AND SDF IN CASES WITH NO FERTILIZATION OR BLASTOCYST FORMATION TO CASES WITH FERTILIZATION AND BLASTOCYST FORMATION RATES HIGHER THAN OR EQUAL TO BENCHMARK RATES

Parameter	ORP (mV/10^6 sperm/ml)	*P*-value	SDF (%)	*P*-value
Fertilization rate (0%) (*n* = 5)	0.83 ± 0.82	0.9593	34.20 ± 14.38	0.0188
Fertilization rate (≥80%) (*n* = 73)	0.59 ± 0.43		19.81 ± 10.80	
Blastocyst formation rate (0%) (*n* = 4)	2.66 ± 2.23	0.0847	31.25 ± 13.84	0.0346
Blastocyst formation rate (≥60%) (*n* = 98)	0.83 ± 2.54		19.87 ± 10.57	

Values reported as mean ± SD.

Benchmark rates according to ESHRE Vienna consensus (*ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine, 2017*).

The significance was calculated using the Mann–Whitney test.

P-values <0.05 indicate statistical significance.

ORP = oxidation-reduction potential; SDF = sperm DNA fragmentation.

blastocyst development: $P = 0.2109$) showed significant differences (ORP: implantation/clinical pregnancy, $P = 0.0004$; live birth, $P < 0.0001$) (SDF: implantation/clinical pregnancy, $P = 0.0012$; live birth, $P = 0.0001$). TABLE 3 reports the mean values of seminal ORP and SDF of cases with no fertilization and no blastocyst formation, compared with those cases where the fertilization and blastocyst formation rates were ≥80% and ≥60%, respectively. While no difference between the groups was found for ORP (fertilization rate $P = 0.9593$; blastocyst formation rate $P = 0.0847$), the differences for SDF were significant (fertilization rate $P = 0.0188$; blastocyst formation rate $P = 0.0346$).

Sperm preparation of the 144 semen samples was performed in 43 (29.9%) samples with standard swim-up and in 101 (70.1%) with density gradient centrifugation. No difference in fertilization (swim-up: $n = 43$; 70.9% versus density gradient centrifugation: $n = 100$; 74.6%; $P = 0.3113$), blastocyst development (swim-up: $n = 42$; 70.3% versus density gradient centrifugation: $n = 96$; 66.8%; $P = 0.4506$), and implantation/clinical pregnancy rates (swim-up: $n = 41$; 35.4% versus density gradient centrifugation: $n = 93$; 28.5%; $P = 0.2983$) as well as for live birth (swim-up: $n = 41$; 39.0% versus density gradient centrifugation: $n = 93$; 24.7%; $P = 0.0945$) was recorded between the two sperm separation techniques.

The correlations between the male parameters analysed in this study are shown in TABLE 4. As expected, significant positive correlations were found between sperm concentration and motility ($r = 0.522$; $P < 0.0001$) and normal morphology ($r = 0.466$; $P < 0.0001$), as well as between normal morphology and motility ($r = 0.496$; $P < 0.0001$). There were also highly significant positive correlations between the percentage of TUNEL-positive spermatozoa and seminal ORP ($r = 0.665$; $P < 0.0001$) as well as male age ($r = 0.328$; $P = 0.0001$). Furthermore, seminal ORP showed a weak, but significant ($r = 0.268$; $P = 0.0012$) positive association with male age. On the other hand, significant negative correlations were found between male age and sperm concentration ($r = -0.215$; $P = 0.0097$), normal morphology ($r = -0.252$; $P = 0.0023$) and motility ($r = -0.271$; $P = 0.0010$), between sperm concentration and ORP ($r = -0.678$; $P < 0.0001$) and the percentage of TUNEL-positive spermatozoa ($r = -0.467$; $P < 0.0001$), between ORP and motility ($r = -0.424$; $P < 0.0001$), and normal morphology ($r = -0.472$; $P < 0.0001$), as well as between the percentage of TUNEL-positive spermatozoa and normal morphology ($r = -0.415$; $P < 0.0001$) and motility ($r = -0.428$; $P < 0.0001$).

Correlations between male age, standard semen parameters, advanced sperm function test (ORP and SDF) and reproductive outcomes (fertilization rate,

TABLE 4 CORRELATION BETWEEN BASIC SEMEN PARAMETERS, RATE OF TUNEL-POSITIVE SPERMATOZOA, SEMINAL ORP AND AGE

Parameter		Concentration (10^6/ml)	Normal morphology (%)	Motility (%)	ORP (mV/10^6/sperm/ml)	TUNEL-positive (%)
Normal morphology (%)	*r*	0.466				
	P	<0.0001				
Motility (%)	*r*	0.522	0.496			
	P	<0.0001	<0.0001			
ORP (mV/10^6 sperm/ml)	*r*	−0.678	−0.472	−0.424		
	P	<0.0001	<0.0001	<0.0001		
TUNEL-positive (%)	*r*	−0.467	−0.415	−0.428	0.665	
	P	<0.0001	<0.0001	<0.0001	<0.0001	
Male age (years)	*r*	−0.215	−0.252	−0.271	0.268	0.328
	P	0.0097	0.0023	0.0010	0.0012	0.0001

Sample size: $n = 144$.

P = *P*-value; *r* = Spearman rank correlation coefficient.

P-values <0.05 indicate statistical significance.

ORP = oxidation-reduction potential; TUNEL = terminal deoxynucleotidyl transferase-mediated dUDP nick-end labelling.

TABLE 5 CORRELATION BETWEEN ADVANCED SPERM FUNCTION TESTS, NORMAL MORPHOLOGY AND REPRODUCTIVE OUTCOMES IN PATIENTS UNDERGOING ICSI TREATMENT

Parameter		ORP (mV/10^6 sperm/ml)	TUNEL-positive (%)	Normal morphology (%)	Fertilization rate (%)	Blastocyst development rate (%)	Implantation / clinical pregnancy rate (%)	Live birth rate (%)
TUNEL-positive (%)	*r*	0.665						
	P	<0.0001						
	n	144						
Normal morphology (%)	*r*	–0.472	–0.415					
	P	<0.0001	<0.0001					
	n	144	144					
Fertilization rate (%)	*r*	–0.267	–0.341	0.156				
	P	0.0012	<0.0001	0.0630				
	n	143	143	143				
Blastocyst development rate (%)	*r*	–0.432	–0.450	0.331	0.034			
	P	<0.0001	<0.0001	0.0002	0.6696			
	n	138	138	138	138			
Implantation / clinical pregnancy rate (%)	*r*	–0.305	–0.280	0.194	0.119	0.187		
	P	0.0003	0.0010	0.0245	0.1712	0.0304		
	n	134	134	134	134	134		
Live birth rate (%)	*r*	–0.366	–0.347	0.196	0.104	0.215	0.780	
	P	<0.0001	<0.0001	0.0232	0.2312	0.0124	<0.0001	
	n	134	134	134	134	134	134	
Male age (years)	*r*	0.268	0.328	–0.252	–0.086	–0.198	–0.239	–0.186
	P	0.0012	0.0001	0.0023	0.3060	0.0215	0.0055	0.0312
	n	144	144	144	134	134	134	134

Advanced sperm function tests include ORP and SDF.

Implantation = cycles with ≥1 gestational sac, clinical pregnancy = cycles with ≥ foetal heart beat and live birth = cycles with ≥1 live baby delivered.

n = sample size; *P* = *P*-value; *r* = Spearman rank correlation coefficient.

P-values <0.05 indicate statistical significance.

ICSI = intracytoplasmic sperm injection; ORP = oxidation-reduction potential; SDF = sperm DNA fragmentation; TUNEL = terminal deoxynucleotidyl transferase-mediated dUDP nick-end labelling.

blastocyst development rate, implantation/clinical pregnancy and live birth) in patients undergoing ICSI treatment are reported in TABLE 5. In the current study, all assisted reproduction parameters (fertilization, blastocyst development, implantation/clinical pregnancy and live birth) were significantly negatively correlated with ORP (r = –0.267, P = 0.0012; r = –0.432, P < 0.0001; r = –0.305, P = 0.0003; and r = –0.366, P < 0.0001, respectively). Similarly, the percentage of TUNEL-positive spermatozoa was significantly negatively associated with these parameters (fertilization: r = –0.341, P < 0.0001; blastocyst development: r = –0.450, P < 0.0001; implantation/clinical pregnancy: r = –0.280, P = 0.0010; live birth: r = –0.347, P < 0.0001, respectively). While male age was not correlated with the fertilization rate, weak but significant negative associations were observed for the blastocyst development rate (r = –0.198; P = 0.0215), implantation/clinical pregnancy (r = –0.239; P = 0.0055) and live birth (r = –0.186; P = 0.0312).

ROC curve analyses for seminal ORP, the percentage of TUNEL-positive spermatozoa and normal sperm morphology were carried out to evaluate the predictive power of seminal ORP, SDF and normal morphology with regard to the benchmark cut-off values for fertilization (≥80%) (FIGURE 2A) and blastocyst development rate (≥60%) (FIGURE 2B), implantation/clinical pregnancy (FIGURE 2C) as well as live birth (FIGURE 2D) after ICSI. The calculations were significant (range of *P*-values: P = 0.0420 to P < 0.0001) for all parameters (ORP, percentage of TUNEL-positive spermatozoa and normal morphology) with respect to all end-points (fertilization and blastocyst development rates, implantation/clinical pregnancy and live birth) except for the prediction of fertilization with normal sperm morphology (AUC 0.572, P = 0.1320) (TABLES 6–9). When comparing the predictive power of the tests by evaluating the areas under the curve (AUC), it appeared that the AUC for normal morphology was the lowest, ranging between 0.572 and 0.732, whereas the AUC for ORP was from 0.652 to 0.794 and that for the percentage of TUNEL-positive spermatozoa from 0.665 to 0.777. When the AUC for these different parameters were directly statistically compared, no differences were found for all end-points (fertilization, blastocyst development, implantation/clinical pregnancy and live birth) except for the comparison TUNEL-positive spermatozoa versus normal sperm morphology for fertilization (P = 0.0400; TABLE 6). Furthermore, while the predictive power for the three parameters tested were well below 70% for fertilization and implantation/clinical pregnancy, it was well above 70% for ORP and the percentage of TUNEL-positive spermatozoa for blastocyst development and live birth. For normal morphology, the predictive power was highest with about 70% for blastocyst development. The relevant calculated cut-off values for seminal ORP predicting good fertilization (>80%), blastocyst formation (>60%), implantation/clinical pregnancy and live birth were ≤0.709, ≤0.530, ≤0.465 and ≤0.393 mV/10^6 sperm/ml, respectively, with an average value of 0.51 mV/10^6 sperm/ml (TABLES 6–9).

Logistic regression with fertilization, blastocyst development, implantation,

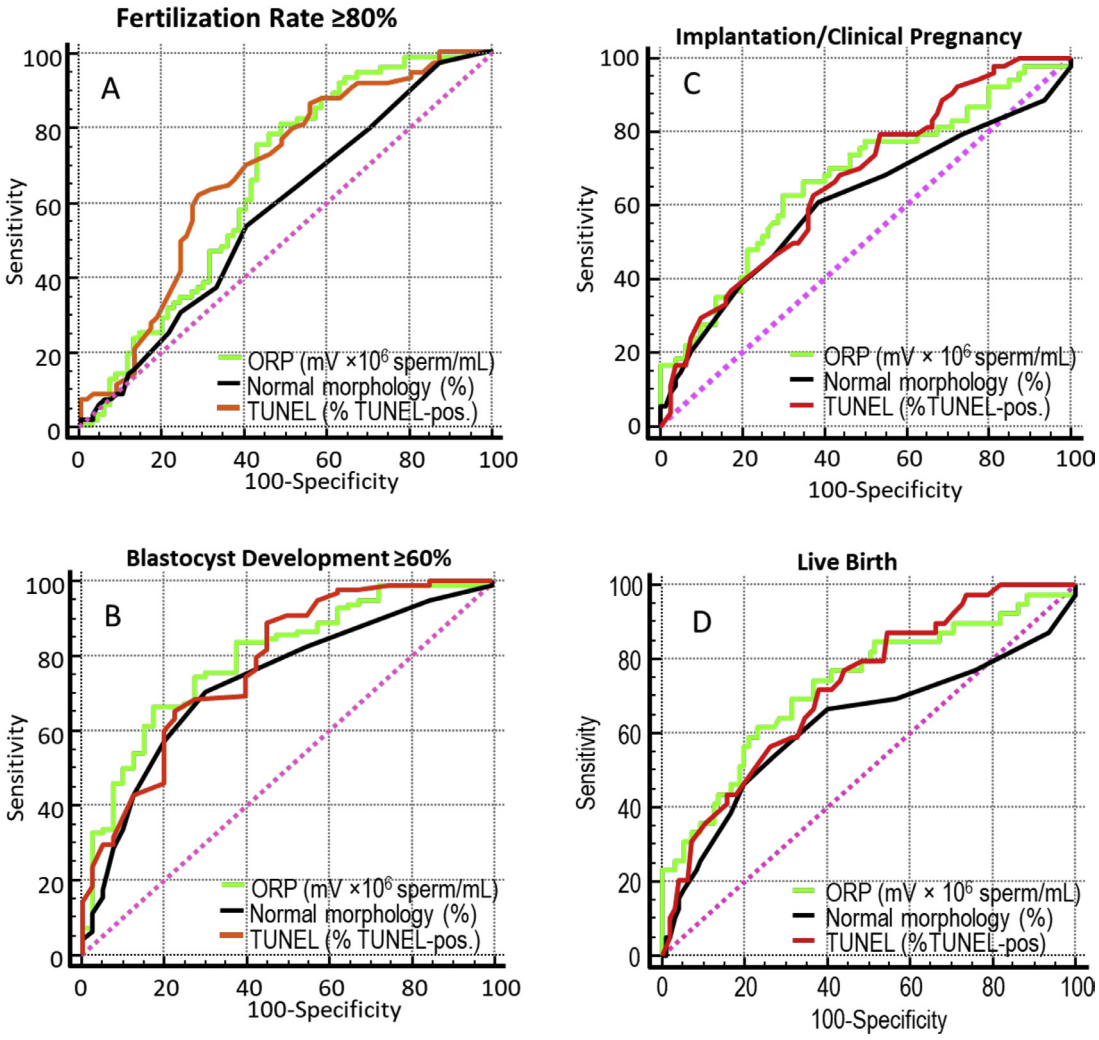

FIGURE 2 Comparison of ROC curves of seminal ORP and the percentages of TUNEL-positive spermatozoa and sperm normal morphology for fertilization rate (≥80%) (A), blastocyst development (≥60%) (B), implantation/clinical pregnancy (C) and live birth (D). The data for the fertilization and blastocyst development rates were categorized according to benchmark classification for ICSI (*ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine, 2017*). Calculations are based on the following numbers: fertilization: *n* = 143, blastocyst development: *n* = 138, implantation/clinical pregnancy: *n* = 134, live birth: *n* = 134. ICSI = intracytoplasmic sperm injection; ORP = oxidation-reduction potential; ROC = receiver operating characteristic; TUNEL = terminal deoxynucleotidyl transferase-mediated dUDP nick-end labelling.

clinical pregnancy and live birth as dependent parameters and high/low seminal ORP (≤0.51 mV/10^6 sperm/ml), primary and secondary infertility, unexplained infertility, polycystic ovaries, endometriosis, tubal factor, myomectomy, ovarian insufficiency and advanced maternal age as independent variables showed overall model fits ($P < 0.0002$) with a significant ($P < 0.0130$) influence of ORP on all reproductive outcome parameters.

TABLE 6 ROC CURVE ANALYSIS RESULTS FOR ORP, NORMAL SPERM MORPHOLOGY AND SDF USING THE BENCHMARK FERTILIZATION RATE (≥80%) AS VARIABLE FOR CATEGORICAL CLASSIFICATION

Variable	AUC	Cut-off	Sensitivity (%)	Specificity (%)	+PV (%)	–PV (%)	*P*-value
ORP (mV/10^6 sperm/ml)	0.652	≤0.709	75.3	57.1	64.7	69.0	0.0012
Morphology (%)	0.572	>4	53.4	60.0	58.2	55.3	0.1320
TUNEL (%)	0.678	≤19	61.6	71.4	69.2	64.1	0.0001
Comp. of AUC	ORP versus TUNEL: ORP versus morphology: TUNEL versus morphology:						0.5151 0.0950 0.0400

P-values <0.05 indicate statistical significance.

AUC = area under the curve; ORP = oxidation-reduction potential; ROC = receiver operating characteristic; SDP = sperm DNA fragmentation; TUNEL = terminal deoxynucleotidyl transferase-mediated dUDP nick-end labelling.

TABLE 7 ROC CURVE ANALYSIS RESULTS FOR ORP, NORMAL SPERM MORPHOLOGY AND SDF USING THE BENCHMARK BLASTOCYST DEVELOPMENT RATE (≥60%) AS VARIABLE FOR CATEGORICAL CLASSIFICATION

Variable	AUC	Cut-off	Sensitivity (%)	Specificity (%)	+PV (%)	–PV (%)	*P*-value
ORP (mV/10^6 sperm/ml)	0.794	≤0.53	66.3	82.5	90.3	50.0	<0.0001
Morphology (%)	0.732	>3	70.4	70.0	85.2	49.1	<0.0001
TUNEL (%)	0.777	<34	88.8	55.0	82.9	66.7	<0.0001
Comp. of AUC	ORP versus TUNEL: ORP versus morphology: TUNEL versus morphology:						0.6794 0.2151 0.3863

P-values <0.05 indicate statistical significance.

AUC = area under the curve; ORP = oxidation-reduction potential; ROC = receiver operating characteristic; SDP = sperm DNA fragmentation; TUNEL = terminal deoxynucleotidyl transferase-mediated dUDP nick-end labelling.

TABLE 8 ROC CURVE ANALYSIS RESULTS FOR ORP, NORMAL SPERM MORPHOLOGY AND SDF USING IMPLANTATION/CLINICAL PREGNANCY AS VARIABLE FOR CATEGORICAL CLASSIFICATION

Variable	AUC	Cut-off	Sensitivity (%)	Specificity (%)	+PV (%)	–PV (%)	*P*-value
ORP (mV/10^6 sperm/ml)	0.680	≤0.465	62.7	71.3	59.6	74.0	0.0002
Morphology (%)	0.614	>4	61.1	62.5	52.4	70.4	0.0278
TUNEL (%)	0.665	≤19	63.0	62.5	53.1	71.4	0.0005
Comp. of AUC	ORP versus TUNEL: ORP versus morphology: TUNEL versus morphology:						0.7332 0.2376 0.3856

Implantation = cycles with ≥1 gestational sac.

P-values <0.05 indicate statistical significance.

AUC = area under the curve; ORP = oxidation-reduction potential; ROC = receiver operating characteristic; SDP = sperm DNA fragmentation; TUNEL = terminal deoxynucleotidyl transferase-mediated dUDP nick-end labelling.

Together with seminal ORP, female age was only included in the stepwise logistic regression model for blastocyst development, implantation/clinical pregnancy ($P = 0.0002$). Among the semen factors, high ORP values proved significant ($P < 0.0018$) in the overall model fit ($P < 0.0045$) for fertilization, implantation/clinical pregnancy and live birth.

Calculation of odds ratios using the cut-off point for a normal ORP of ≤0.51 mV/10^6 sperm/ml obtained in this study and the various reproductive end-points (fertilization, blastocyst development, implantation/clinical pregnancy and live birth) resulted in significantly higher odds for patients achieving ≥80% fertilization (OR 0.4651; 95% CI 0.2385–0.9070; $P = 0.0247$), ≥60% blastocyst development (OR 0.1287; 95% CI 0.0517–0.3203; $P < 0.0001$), implantation/clinical pregnancy (OR 0.3163; 95% CI 0.1535–0.6517; $P = 0.0018$) and live birth (OR 0.2299; 95% CI 0.1005–0.5260; $P = 0.0005$), if the ORP was low, i.e. ≤0.51 mV/10^6 sperm/ml (TABLE 10). If the previously published cut-off value of 1.34 mV/10^6 sperm/ml (*Agarwal et al., 2019*) was used, the results were only significant for fertilization and blastocyst development, but not for clinical pregnancy and live birth (data not shown).

A similar trend was observed in the odds ratios when the male age was taken into consideration as confounding factor (TABLE 11). It was also obvious that older men (≥37 years) with high ORP (>0.51 mV/10^6 sperm/ml) not only have a lower chance of fertilizing oocytes, but also a lower chance of good (≥60%) blastocyst development. Consequently, this will

TABLE 9 ROC CURVE ANALYSIS RESULTS FOR ORP, NORMAL SPERM MORPHOLOGY AND SDF USING LIVE BIRTH AS VARIABLE FOR CATEGORICAL CLASSIFICATION

Variable	AUC	Cut-off	Sensitivity (%)	Specificity (%)	+PV (%)	–PV (%)	*P*-value
ORP (mV/10^6 sperm/ml)	0.728	≤0.393	61.5	76.8	52.2	83.00	<0.0001
Morphology (%)	0.621	>4	66.7	60.0	40.6	81.4	0.0420
TUNEL (%)	0.723	≤19	71.8	62.1	43.8	84.3	<0.0001
Comp. of AUC	ORP versus TUNEL: ORP versus morphology: TUNEL versus morphology:						0.9100 0.1183 0.1504

Live birth = cycles with ≥1 live baby delivered.

P-values <0.05 indicate statistical significance.

AUC = area under the curve; ORP = oxidation-reduction potential; ROC = receiver operating characteristic; SDP = sperm DNA fragmentation; TUNEL = terminal deoxynucleotidyl transferase-mediated dUDP nick-end labelling.

TABLE 10 ODDS RATIOS FOR VARIOUS REPRODUCTIVE OUTCOME POINTS USING A CUT-OFF VALUE FOR ORP OF 0.51 MV/10⁶ SPERM/ML

Rate	ORP		Numbers per group (%)	Odds ratio(95% CI)	*P*-value
	≤0.51 mV/10⁶/ml	>0.51 mV/10⁶/ml			
Fertilization					
<80%	28	42	70 (49.0%)	0.4651 (0.2385 to 0.9070)	0.0247
≥80%	43	30	73 (51.0%)		
	71	72	143		
Blastocyst development					
<60%	7	33	40 (29.0%)	0.1287 (0.0517 to 0.3203)	<0.0001
≥60%	61	37	98 (71.0%)		
	68	70	138		
Implantation / clinical pregnancy					
No	31	49	80 (59.7%)	0.3163 (0.1535 to 0.6517)	0.0018
Yes	36	18	54 (40.3%)		
	67	67	134		
Live birth					
No	38	57	95 (70.9%)	0.2299 (0.1005 to 0.5260)	0.0005
Yes	29	10	39 (29.1%)		
	67	67	134		

Implantation = cycles with ≥1 gestational sac, clinical pregnancy = cycles with ≥ foetal heart beat and live birth = cycles with ≥1 live baby delivered.

P-values <0.05 indicate statistical significance.

ORP = oxidation-reduction potential.

result in a lower chance of implantation/clinical pregnancy and live birth than in men with low ORP or young men. Overall, younger men have higher chances of a successful reproductive outcome.

DISCUSSION

Although the WHO has standardized the procedures for conventional standard semen analysis (*World Health Organization, 2010*, *2021*), it cannot predict male fertility and fertilizing potential of spermatozoa (*Nagler, 2011*; *Wang and Swerdloff, 2014*). Because the fertilization process is multifactorial (*Henkel et al., 2005*), any given test can only provide a limited probability for successful fertilization and even less forecast a positive reproductive outcome. Consequently, conventional semen parameters provide only a limited prognosis for adequate sperm function and do not identify the cause of the infertility in about 30–50% of cases (*Chehab et al., 2015*; *Jungwirth et al., 2015*). Hence, these cases remain idiopathic or unexplained and frequently do not receive the necessary treatment. Due to the problems of standard semen analysis in predicting male fertilizing potential, additional sperm function tests including tests for sperm DNA damage and oxidative stress, i.e. tests for SDF and ROS, have been developed.

While the clinical value of SDF testing is gradually being acknowledged and recommended (*Tharakan et al., 2022*; *World Health Organization, 2021*), tests for oxidative stress are still regarded as research tests. Traditionally, seminal oxidative stress is measured by direct determination of the ROS using luminescent probes such as luminol or lucigenin (*Aitken et al., 1992*). Alternatively, the determination of the total antioxidant capacity (*Sharma et al., 1999*) has been suggested as an indirect measure for oxidative stress. However, these techniques suffer from high variation in results and are not generally accepted for routine use. A novel galvanostatic technique measures the balance between oxidation and reduction, the ORP, and has been shown to distinguish between sperm donors and infertile patients (*Agarwal et al., 2016*). However, it is still considered experimental, as there is insufficient evidence to correlate seminal ORP with reproductive outcomes. To the best of our knowledge, the current study is the first presenting evidence for the predictive value of seminal ORP measurement with regard to reproductive end-points, namely fertilization, blastocyst development, implantation/clinical pregnancy and live birth after ICSI.

As expected, seminal ORP was significantly negatively correlated with sperm concentration, motility and normal morphology (*Agarwal et al., 2018*; *Cicek et al., 2021*) and significantly positively with male age (*Nago et al., 2021*) and SDF (*Arafa et al., 2019*). While the former associations indicate the negative impact of seminal oxidative stress on sperm functions, the latter associations, although only weak, are in line with the age-related increase in seminal ROS concentrations (*Cocuzza et al., 2008*). Yet, logistic regression revealed that ORP is independent from female and other semen parameters. This not only explains the significant associations between male age and the reproductive outcomes, but also the observation that older men (≥37 years) with high seminal oxidative stress (>0.51 mV/10⁶ sperm/ml) have lower

TABLE 11 ODDS RATIOS FOR VARIOUS EMBRYOLOGICAL END-POINTS USING A CUT-OFF VALUE FOR ORP OF 0.51 MV/10^6 SPERM/ML AND MALE AGE AS CONFOUNDING FACTOR

Male age	Reproductive outcome parameter	ORP cut-off				Odds ratio for age group	Overall odds ratio(95% CI)	*P*-value
		≤0.51 mV/10^6/ml		>0.51 mV/10^6/ml		Total no. of cases		
	Fertilization ≥80%	No	Yes	No	Yes			
<37 years		14	26	14	14	0.5385	0.5016 (0.2545 to 0.9889)	0.04667
≥37 years		14	17	28	16	0.4706		
Total no. of cases		28	43	42	30	143		
	Blastocyst development ≥60%	No	Yes	No	Yes			
<37 years		3	37	7	21	0.2432	0.1447 (0.0572 to 0.3657)	0.00001
≥37 years		4	24	26	16	0.1026		
Total no. of cases		7	61	33	37	138		
	Implantation / clinical pregnancy	No	Yes	No	Yes			
<37 years		19	21	19	9	0.4286	0.3272 (0.1578 to 0.6786)	0.0024
≥37 years		12	15	30	9	0.2400		
Total no. of cases		31	36	49	18	134		
	Live birth	No	Yes	No	Yes			
<37 years		23	17	23	5	0.2941	0.2370 (0.1028 to 0.5461)	0.0005
≥37 years		15	12	34	5	0.1838		
Total no. of cases		38	29	57	10	134		

Implantation = cycles with ≥1 gestational sac, clinical pregnancy = cycles with ≥ foetal heart beat and live birth = cycles with ≥1 live baby delivered.

P-values <0.05 indicate statistical significance.

ORP = oxidation-reduction potential.

chances of their spermatozoa resulting in successful reproductive outcome than men of the same age group with low seminal ORP values (≤0.51 mV/10^6 sperm/ml). This further implies that an antioxidant treatment or lifestyle changes leading to a decrease in oxidative stress may result in increased fertilization and improved blastocyst development rates.

ROC curve analyses for normal sperm morphology, SDF and seminal ORP were performed in this study for fertilization, blastocyst development, implantation/clinical pregnancy and live birth. All tested sperm/semen parameters significantly predicted the reproductive outcome parameters included in this study with predictive powers between 61.4% (normal morphology for implantation/clinical pregnancy) and 79.4% (ORP for good blastocyst development). Notably, normal sperm morphology showed the worst predictive power for all classification variables. This might be because in this study all patients were treated with ICSI, where a selection of the best morphologically appearing vital spermatozoa for injection is performed. When the AUC were statistically compared, no differences between the AUC of the sperm/semen parameters, except between SDF and morphology for fertilization, were observed. In fact, the AUC of SDF and ORP were comparable with those reported for SDF in previous studies (*Cissen et al., 2016*; *Muratori et al., 2015b*; *Wiweko and Utami, 2017*).

Interestingly, while cut-off values for SDF and normal sperm morphology were calculated for all reproductive outcome points in the range of what was previously published, between 19% and 36.5% for SDF and 4% for normal morphology, respectively, the cut-off point for ORP with an average of 0.51 mV/10^6 sperm/ml was markedly lower than those (1.34, 1.36 and 1.38 mV/10^6 sperm/ml) published previously (*Agarwal et al., 2017*, *2019*; *Arafa et al., 2018*). A possible explanation for this discrepancy could be that these values were not calculated by using reproductive outcomes in an assisted reproduction programme, but by distinguishing between men with normal/abnormal semen parameters and donors/patients, respectively. However, when considering the clinical value of a specific test in andrological diagnostics, reproductive outcome parameters should be used. Therefore, a lower cut-off value for seminal ORP than the previously published ones seems reasonable and a cut-off of ≤0.51 mV/10^6 sperm/ml is proposed. However, at this stage, it must also clearly be stated that this value still needs to be validated in larger, more controlled studies. This value might also differ for IVF or for intrauterine insemination (IUI) because for IVF and IUI all sperm functions have to be in the optimum range to achieve a clinical pregnancy and live birth.

In many studies trying to evaluate sperm functional tests, fertilization is used as the primary reproductive end-point without further exploring possible adverse effects in early embryo development, implantation/clinical pregnancy and live birth. However, considering that spermatozoa with DNA damage can still fertilize oocytes (*Henkel et al., 2004*), this might not be the best end-point to assess sperm functional capability. In addition, one has also to consider that besides the fertilizing capacity of spermatozoa, blastulation depends on the quality of the oocyte as well as the culture conditions. Moreover, the occurrence of clinical pregnancy and live birth depend on a good embryo transfer, endometrial receptivity as well as on female variables such as pre-eclampsia, systemic

diseases or preterm labour. Based on this, it is obvious that the AUC and the odds ratios diminished steadily from fertilization to live birth. This can clearly be seen in the current results. Therefore, later reproductive end-points were used in this study.

Because no difference between the AUC for ORP and SDF was observed, the question arises as to which assay might be clinically most suitable. In this study, the association between SDF and seminal oxidative stress measured as ORP was confirmed (*Homa et al., 2019*), but it should not be forgotten that DNA damage can also occur due to defective or failing DNA repair mechanisms (*Puzuka et al., 2021*). Hence, both tests complement each other. However, when considering time, labour and cost, these factors seem to be in favour of the determination of ORP. As reported previously (*Rex et al., 2017*), the TUNEL assay is labour-intensive. In contrast, the analysis of seminal ORP requires only about 4 min following liquefaction. Hence, the result can be discussed with the patient on the same day alongside possible treatment options.

Although this study clearly shows the impact of seminal oxidative stress on reproductive outcome in a wider range of male patients, the study has limitations. First, the use of ICSI might have contributed to the fact that the calculated cut-off value of ORP was lower than the previously published values because sperm functions such as motility or acrosome reaction did not play a role in the fertilization process. In contrast, for IUI and IVF, these sperm functions play an essential role as capacitation has to be triggered by a small amount of ROS. Therefore the cut-off value of ORP may be different for IUI or IVF. Second, the use of a fluorescence microscopic TUNEL assay limited the number of spermatozoa that were evaluated. Third, this study was conducted using a non-select cohort of patients in whom seminal ORP was determined and related to reproductive outcomes. Further prospective studies need to confirm the current findings to see whether the management of patients on the basis of the ORP results can improve clinical outcomes in infertile patients.

In conclusion, this is the first study showing the impact of seminal ORP on fertilization, blastocyst development, implantation/clinical pregnancy and live birth as reproductive outcome parameters with an average cut-off value of 0.51 mV/10^6 sperm/ml in an ICSI programme. However further evaluation, not only by carefully establishing a physiological range of ORP for normal sperm functions, but also by conducting larger studies including IVF and IUI, are necessary to clearly establish the predictive capabilities of ORP in andrological diagnostics for assisted reproduction. An early and reliable detection of seminal oxidative stress can help to further personalize ART treatment in male infertility patients and guide clinicians in counselling patients. Such an approach could then lead to an increase in successful fertility treatments or the use of less invasive approaches, thereby leading to better cost-effectiveness in healthcare systems. However, at this stage, it must also be pointed out that the measurement of seminal ORP cannot be offered to patients with severe oligozoospermia or azoospermia. Based on these findings, it can be concluded that ORP is a useful parameter to be incorporated in the evaluation of male infertility. In order to uncover the impact of seminal ORP on other sperm functional parameters, which play an essential role in natural conception as well as for IVF or IUI, relevant studies are under way.

DATA AVAILABILITY

The data underlying this article will be shared on reasonable request to the corresponding author.

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