

the spermatozoa to induce oocyte activation in the mouse model was confirmed. One man had a familial case of globozoospermia confirmed by TEM while another man did not provide a test specimen due to religious beliefs. The safety of the sperm permeabilization treatment by SLO was assessed by TUNEL resulting in 8.4% DNA fragmentation in treated spermatozoa versus 10.2% in the control. The amount of PLC $\zeta$  in spermatozoa of these infertile men ranged between 0-14.4% while 37.0-80.7% in men with proven fertility ( $P = 0.0001$ ; Table).

**Table:** Comparison of PLC $\zeta$  presence in known fertile and infertile men.

No. of spermatozoa	Control $n = 10$	Study group $n = 8$
Positive (PLC $\zeta$ )	5213 (60.0)*	283 (4.9)*
Negative	3473	5510
	8686	5793

\* $P = 0.0001$

Study specimens had an average concentration of  $78.2 \pm 49 \times 10^6/\text{ml}$  with a motility of  $43.7 \pm 15\%$ , and normal morphology of  $1.7 \pm 1\%$ . Oocyte activation yielded 51.9% (41/79) 2PN. The cleavage rate was 87.7% with the mean number of blastomeres being  $7.2 \pm 1$  on day3, and a mean fragmentation rate of  $8.4\% \pm 4$ . Conceptuses were available for transfer in all cases with a mean of 1.7 embryos. Seven (38.9%) positive bhCG of which 3 (16.7%) progressed to clinical pregnancies and one couple delivered a healthy baby boy.

**Conclusions:** Screening for presence and expression of SCF allowed the correct diagnosis of individuals incapable of fertilization and provide an indication to treatment by assisted oocyte activation. We were able to quantify a defective expression of SCF in the study group. Utilization of a recombinant PLC $\zeta$  or a metabolome of the cascade such as IP3 may provide a more physiological way to correct for fertilization failure or to normalize SCF deficiency.

#### P-596 Application of an optimised two tailed comet assay on human cumulus cells

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**Introduction:** Research has indicated that cumulus cell DNA integrity may be associated with assisted reproductive technology (ART) outcomes, potentially serving as a marker of oocyte competence. Studies have utilised the alkaline comet assay and TUNEL assay to assess the levels of DNA damage in cumulus cells (CCs). However, these methods are not able to distinguish between double stranded DNA breaks (DSBs) and single stranded DNA breaks (SSBs). DSBs are generally considered to be more lethal than SSBs. Most recently, Enciso et al demonstrated that the two-tailed comet assay applied to human spermatozoa was able to provide an evaluation of the presence of DSBs and SSBs in the same cell. It may also be useful to distinguish the different types of DNA fragmentation in cumulus cells which may provide a more refined evaluation of genetic damage and produce a useful non-invasive method to assess oocyte quality. We have optimised the two-tailed comet assay to assess SSBs and DSBs in human CCs and conducted a pilot study to determine if the different types and grades of DNA fragmentation in CCs could be associated with ART outcomes.

**Material and Methods:** Cumulus cells were collected from 41 women undergoing ART at a single fertility clinic and the CCs were pooled together for each woman in the pilot study. The average maternal age was 36.3 years (age range: 24 – 48 years). Intracytoplasmic sperm injection (ICSI) was used for fertilisation in all cases. The optimised two tailed comet assay was applied to each sample of pooled CCs with the types and grades of DNA fragmentation visually scored. The nucleoids are graded 0-3 with grade 0 having no comet tail observed and grade 3 having a distinct and long comet tail observed. A comet tail observed will indicate either the presence of DSBs or SSBs. ANOVA was utilised to compare the age groups and BMI groups of the women involved in the pilot study. Bivariate correlation analyses were performed for 5 subgroups analyses of 41 women, 39 women with known BMI, 29 women with data on day 2 embryos, 28 women with data on day 3 embryos and 37 women with data on pregnancy outcomes. Linear regression analysis was employed to correlations observed which were statistically significant ( $p < 0.05$ ) to examine if any of the observed variables could predict clinical outcomes.

**Results:** A summary of the results from the 5 subgroup analyses suggested that CCs with DSBs only predict the number of immature or poor quality oocytes, number of oocytes fertilised and number of zygotes formed ( $p < 0.05$ ). CCs with grade 2 DSBs and grade 3 SSBs consistently predicted the percentage of mature (MII) oocytes collected ( $p < 0.05$ ). The average DNA fragmentation score per CC was predictive of the number of oocytes fertilised and the number of zygotes formed ( $p < 0.05$ ). Age and BMI also appeared to affect the types and grades of DNA fragmentation in CCs ( $p < 0.05$ ).

**Conclusions:** There is strong evidence that the types and grades of DNA damage in CCs influence the quality of the oocytes they support, ultimately affecting ART outcomes. The two tailed comet assay applied to CCs has great potential for offering an accurate and inexpensive method of evaluation of the genomic integrity of cumulus cells. This will be of strong clinical interest, potentially assisting in the identification of viable oocytes and predicting ART outcomes.

#### P-597 Optoelectronic tweezers for use to identify and retrieve viable, optimal quality non-motile human sperm for ICSI: feasibility and biosafety studies

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**Introduction:** IVF/ICSI success rates remain suboptimal and are limited, especially when non-motile sperm are used, by our inability to reliably predict sperm quality before injection. We recently introduced a novel light-based dielectrophoresis (DEP) technology/assay, termed **Optoelectronic Tweezers (OET)**, which can be used with unwashed human semen to distinguish viable from non-viable non-motile individual sperm. Sensitivity and specificity is comparable (and possibly superior) to Trypan Blue. DEP fields are projected onto a slide containing a suspension of mixed sperm. Viable sperm, which preserve a net-dipole moment, are spontaneously attracted to the OET-DEP field, and non-viable sperm are repulsed, all regardless of the presence of motility. By moving the projected OET-DEP field, individual non-motile sperm can also be moved about the field, for retrieval. In parallel work using a preimplantation mouse embryo model, we have shown that OET-DEP also predicts both absolute embryo viability and developmental stage (independent of visible morphometric indices).

In this work, we hypothesize that magnitude of response to an OET-DEP field could predict relative viability (quality) among human sperm, and herein, assess the spectrum of response among viable sperm. We also assess the biosafety of OET-DEP using alkaline COMET DNA fragmentation assay of assayed human sperm, and, we use a preimplantation mouse embryo model to assess whether exposure of embryos to OET-DEP interferes with embryo viability and pup health following uterine transfer to surrogate females.

#### Material and Methods:

**Human sperm:** Aliquots of sperm from 6 healthy subjects underwent OET and Trypan Blue viability assay (Trypan assay = reference assay). The maximum speed at which sperm could be pulled (if attracted; assigned a + velocity value), or pushed (if repulsed; assigned a -velocity value), was recorded.

Separate OET-exposed and non-exposed aliquots underwent alkaline COMET assay. Negative (unexposed) and positive (peroxide-treated) assay-controls were used. A dose-response effect of OET voltage was assessed. Percent DNA in Tail (PDIT), Tail Moment (TM), and Olive Moment (OM) were measured.

**Mouse Embryo:** B6D2F1 morphometrically-indistinguishable early-blastocyst embryos ( $N = 384$ ) were used. Two randomly selected cohorts ( $N = 192, 192$ ) underwent OET assay versus no OET assay, followed by (blinded) oviduct transfer to recipient CD1 females (16 embryos/recipient,  $N = 12, 12$ ). Birth rates, birth-weight, and pup attainment of developmental milestones were assessed (two-tailed  $\alpha = 0.05$ ,  $b = 0.90$  to detect birth rate differences  $> \pm 12\%$ ,