Novel Therapies To Protect Sperm Quality In ART

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The manipulation of sperm for assisted reproductive technology (ART) results in a significant decrease in sperm quality, especially higher levels of DNA damage. Elevated levels of DNA damage in sperm have been linked to poor-quality embryos and increased pregnancy loss after ART. Utilizing the novel therapeutic BGP-15 we have previously shown that treating the whole ejaculate prior to and during cryopreservation increases sperm quality compared with frozen/thawed untreated samples. In this study, we investigated the potential of this BGP-15 therapy to preserve sperm quality during semen washing prior to IVF/ICSI.

Donated human semen specimens (N=41), excess to ART, were treated with 10 µM BGP-15, and sperm motility and DNA damage (DNA fragmentation and oxidation) were first examined to understand the effect of incubation time in whole semen. Samples were then washed using different clinical sperm washing methods: simple wash, swim-up and density gradient centrifugation (DGC); and purified sperm underwent assessment for motility, vitality, ROS levels, mitochondrial membrane integrity, mitochondrial membrane potential, and DNA damage. Statistical analysis was two-tailed paired Student's T test when comparing between two groups, or repeated measures Two-Way ANOVA with post-hoc paired t-tests when comparing between three groups.

Semen samples incubated with BGP-15 demonstrated 15% improved sperm motility ($p=0.002$) and 57% reduced oxidative DNA damage levels ($p=0.03$).

Comparison between the untreated groups for each washing method, showed that simple washed samples had the highest sperm recovery rate, which was 55% and 21% higher than DGC ($p<0.005$) and swim-up ($p<0.0005$), respectively. Additionally, sperm recovered by swim-up had 18% and 27% increased vitality compared to DGC ($p<0.001$) and simple wash ($p<0.0001$), respectively. Swim-up sperm also presented DNA oxidation levels reduced by 40% and 76% when compared to simple wash ($p=0.01$) and DGC ($p<0.0001$), respectively. However, swim-up sperm also had the lowest mitochondrial membrane potential, that was 28% lower than simple wash and DGC ($p<0.03$).

Comparison between untreated and BGP-15 treated groups for each sperm washing method showed that DGC sperm had 11% increased mitochondrial membrane potential after BGP-15 treatment ($p=0.0006$). Moreover, BGP-15 treatment only reduced sperm DNA fragmentation in washed samples (22% reduction, $p=0.03$), but it lowered DNA oxidation by 48% in washed ($p=0.002$), by 42% in swim-up ($p=0.04$) and by 29% in DGC ($p<0.0001$) sperm.

We further tested the safety of sperm treatment with BGP-15 in a mouse model and analyzed its effect on IVF, pre-implantation embryo development, embryo cryopreservation and thawing. After surgical embryo transfer, surrogate female mice were assessed for pregnancy success, gestation length and litter size. Litters were also assessed for growth trajectory, righting reflex and survival to weaning at 21 days old. There were no differences between untreated and BGP-15 groups in any measurement analyzed, indicating there was no detectable effect of *in vitro* BGP-15 treatment of sperm on embryo, pregnancy or offspring outcomes (n=6 males/pools of embryos/surrogate females/litters per treatment group; $p>0.1$).

Our findings indicate that the utilization of BGP-15 during semen preparation can protect and improve on its quality. Our preclinical models further highlight that the addition of BGP-15 could be used clinically to improve the outcomes of patients undergoing ART.