P-479: PRESSURE TRIGGERED ACTIVATION OF TOLERANCE (PTAT)-PRECONDITIONING OF SPERMATOZOA HAS A POSITIVE IMPACT ON POST-THAW RECOVERY RATE OF CRYOPRESERVED HUMAN SEMEN

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INTRODUCTION

Although human semen cryopreservation is a routinely applied technology, sperm recovery is rather variable and difficult to predict. On average, about 50% of spermatozoa survive freezing and thawing[1-3], but post-thaw motility varies between the wide range of 25 and 75 percent[4-6]. The prerequisite for sperm bank donors is usually a recovery rate of at least 40-50% of basal motility[4;5;7], but efficient cryopreservation is also a clear expectation of patients attending to fertility preservation or IVF. Albeit there is a huge effort in identifying biological factors predictive for semen freezability[4;8-15], cryosurvival depends not only on basic semen parameters, but also on the overall condition of the cells at the time of freezing, and the cryopreservation procedure itself.

PTAT (formerly known as HHP, high hydrostatic pressure) treatment prior to freezing procedures has been successfully applied to promote cryosurvival in several mammalian species (bovine, porcine, ovine, mouse) and cell types (sperm cells, oocytes, embryos, etc.)[16].

Based on our recent animal studies PTAT technology has improved cell cryotolerance and fertility rate (*Figure 1*; *Figure 2*).

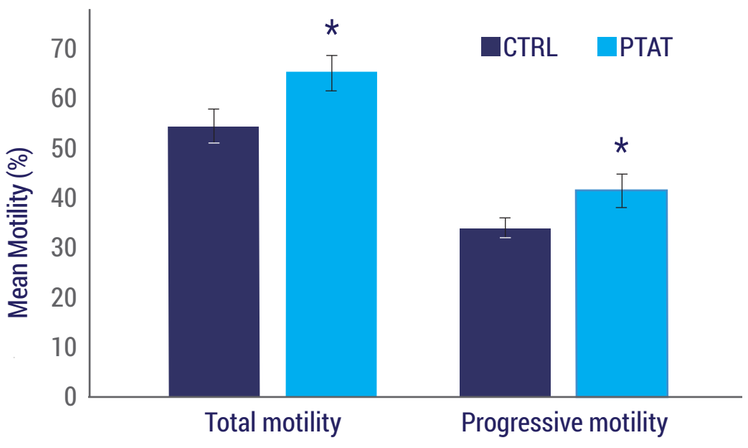


*Figure 1: Total and progressive motilities measured in treated (PTAT) and control (CTRL) bull semen (n=131 ejaculates; 90 bulls; mean ± SEM; *p<0.05).*

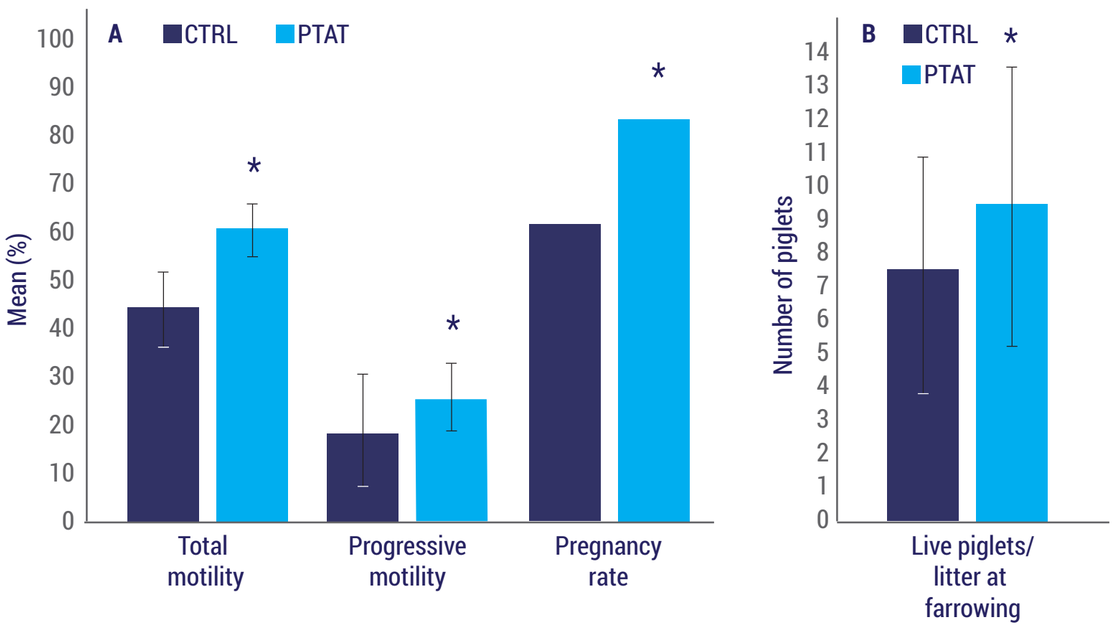


*Figure 2: Total and progressive motility, pregnancy rate (A), and litter size (B) for treated (PTAT) and control (CTRL) boar semen (102 inseminations; 672 live piglets; mean ± SD; *p<0.05).*[17]

STUDY DESIGN, PARTICIPANTS, MATERIALS AND METHODS

Semen samples of 26 Caucasian men were included in this proof-of-concept case-control study, who all met the inclusion criteria (18-50 years of age, semen volume of at least 3 ml, basal total motility at least 40%, no existing andrological disease or any symptoms of urogenital infection).

After the initial semen analysis according to WHO directions[1], PTAT preconditioning of the case samples was performed on a programmable GBOX2010 device by Applied Cell Technology, Budapest, Hungary (*Figure 3*), applying 100-500 bar pressure for 30-90 minutes. After equilibration all samples were frozen with a standard slow freezing protocol.

After thawing, samples were centrifuged and resuspended in SpermWash® (*Gynotec, Malden, The Netherlands*), and total, progressive and nonprogressive motilities were defined by CASA (*Microptic, Barcelona, Spain*). Measurements were performed 30, 60, 120 and 240 minutes after thawing (*Figure 4*). All data are presented as means ± SEM.

Statistical analysis was performed using the Student's paired t-test and the Bland-Altman plot. P values of <0.05 were considered to be statistically significant.

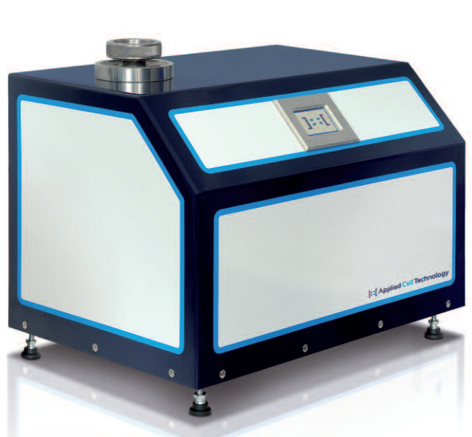


Figure 3: Computer controlled high hydrostatic pressure device (GBOX2010)

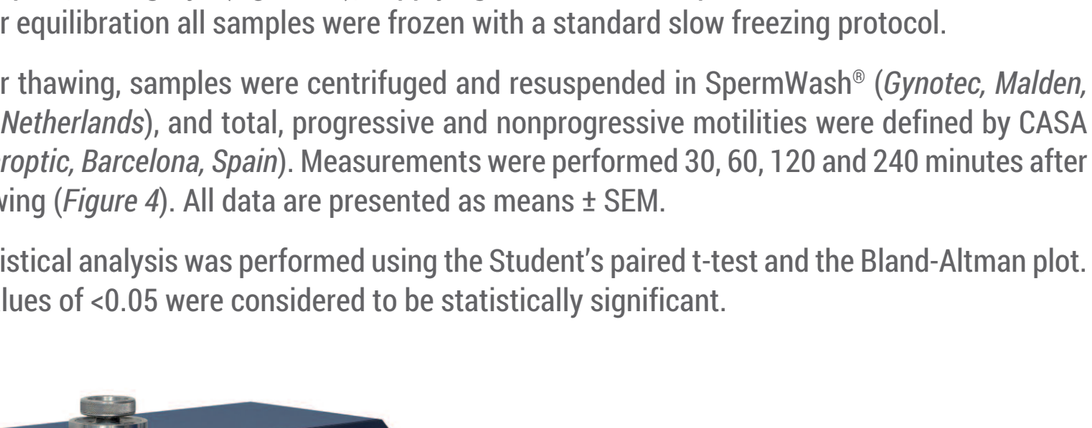


Figure 4: General process of sperm freezing including PTAT preconditioning

MAIN RESULTS AND THE ROLE OF CHANCE

We have set up several PTAT-parameters, but pressures below 200 bar and above 300 bar were shortly rejected, as no benefits seemed to be expected (data not shown), and the pressure tolerance matrix was reduced to three treatment groups (200 bar / 60 min, 200 bar / 90 min, 300 bar / 60 min).

The highest effect size was at 200 bar / 90 min PTAT-parameters resulted in statistically significant increase in total (9.96% ± 3.33) and progressive (6.70% ± 1.99) motility (p=0.017 and p=0.036; respectively), compared to the paired controls (*Figure 5, Figure 6*). This beneficial effect was long-lasting and the major difference was observed at 60 minutes after thawing.

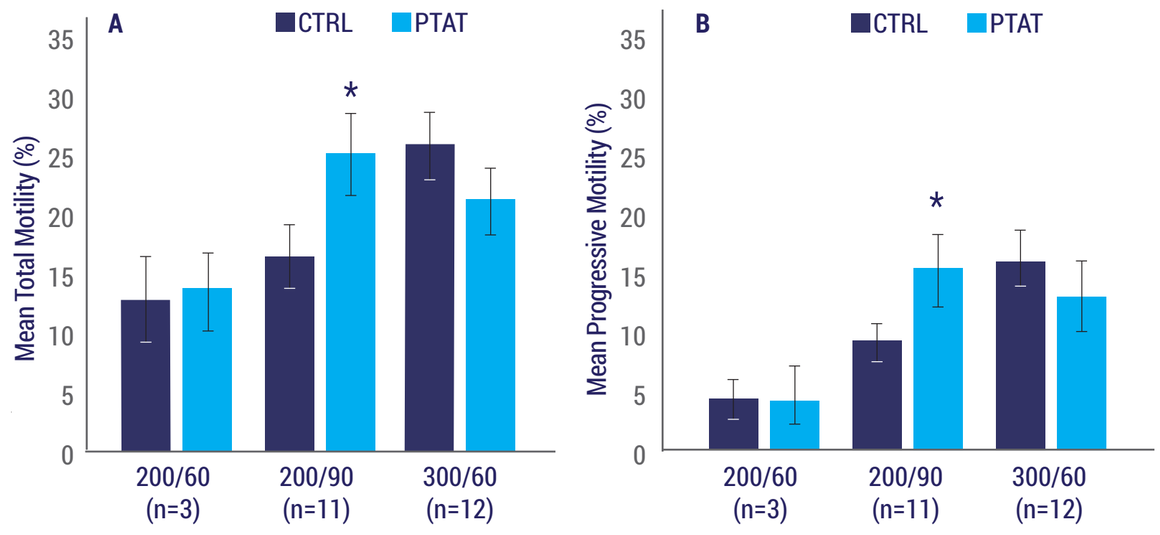
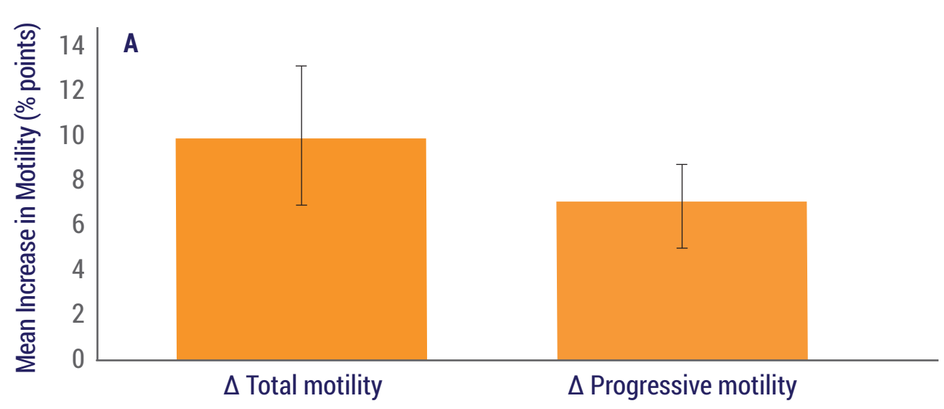


*Figure 5: Mean total (A) and progressive (B) motilities in the three PTAT-treatment groups (PTAT), compared to paired controls (CTRL) (mean ± SEM; *p<0.05).*

CONCLUSIONS

Our proof-of-concept study based on the analysis of normozoospermic sperm samples suggests that PTAT is able to improve human sperm cell survival after slow freezing. In order to prove the safety and efficacy of the method, flow cytometry measurements (vitality, DNA-integrity) and morphological evaluations are currently in progress. The novel observations described herein represent a good basis for further, in-depth examinations on larger, or even non-normozoospermic populations.



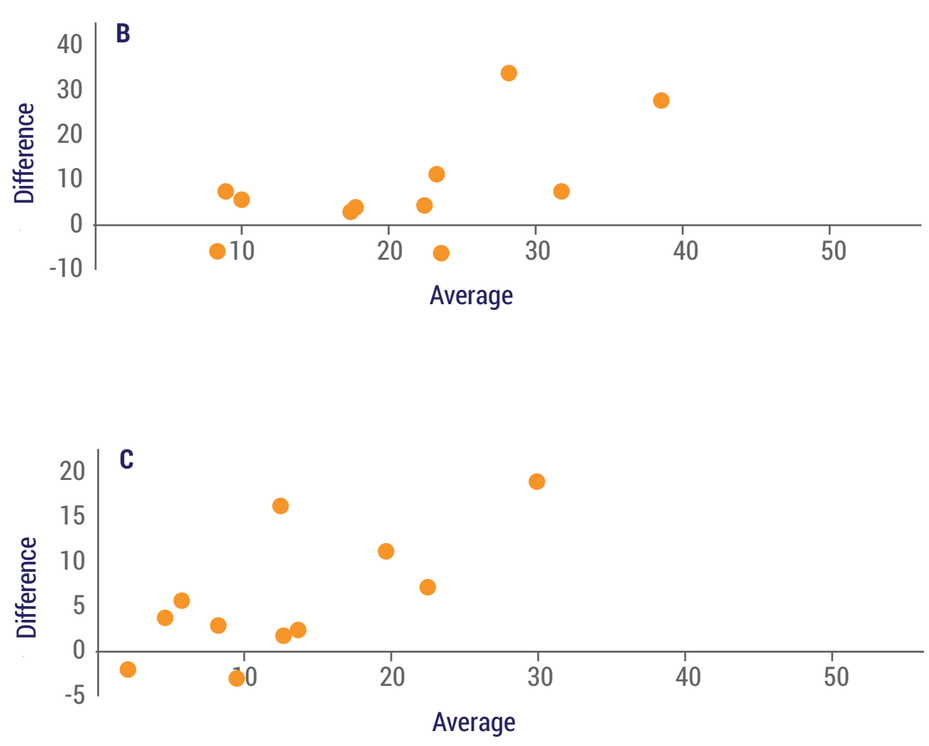


Figure 6: Mean increase in post-thaw total and progressive motility (A) ± SEM after PTAT preconditioning at 200 bar for 90 min, compared to paired control (n=11). Bland-Altman plot of changes in post-thaw total (B) and progressive (C) motility, in PTAT-samples compared to paired controls (% points).

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