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Human sperm storage without freezing in electrolyte-free monosaccharide solutions

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1. Purpose

Development of an effective method of sperm preservation for several weeks without freezing in liquid nitrogen will provide safe transporting and facilitate planning for medical procedures through the infertility treatment programs. Sperm is known to be capable of maintaining viability in cauda epididymis for several days or weeks at the temperature slightly below body temperature, therefore one of possible ways to preserve sperm apart from freezing is partial imitation of conditions in epididymis.

K. Saito et al. in 1996 proposed a novel way of human sperm preservation at +4 ^{o}C in salt-free water glucose solution, which appears to be the most effective technic of preservation without freezing. At the same time there are other simple sugars present in the epididymal fluid at concentrations sometimes significantly higher than those of glucose level. In fact aldose reductase and sorbitol dehydrogenase, converting glucose to sorbitol and then to fructose are highly active in epithelial cells. These two enzymes are also present in epididimosomes that enter the epididymal lumen in an apocrine mode (Frenette et al., 2006). Therefore we suggested that keeping sperm in electrolyte-free fructose or even sorbitol (sugar alcohol) solutions may be consistent with the natural equilibrium of sperm in epididymis.

2. Methods and Materials

41 samples of capacitated sperm remained after in vitro fertilization within infertility treatment programs were used in experiment. Samples were selected randomly, irrespective of baseline characteristics. Sperm was prepared using SupraSperm® System kit (MediCult) according to instruction given. Water solutions of 3% BSA (bovine serum albumin) and around 0.33 M glucose (Saito et al., 1996; Kanno et al., 1998), sorbitol and fructose respectively were used as preservatives. Solutions were adjusted to 320 mosmol/kg with monosaccharide or polyol. 30 µl pellet of each sperm sample was diluted in 2 ml of preservative. Tubes with samples were kept at +4 ^{o}C for two weeks. After two weeks samples were centrifuged and preservative was removed. Sperm was diluted to 0.1 mln/ml and incubated in FertiCult IVF medium (FertiPro) for 2 hours at +37 ^{o}C in an atmosphere of 5% CO_2, 5% O_2 and 90% N_2. Sperm motility was estimated according to WHO recommendations. The rate of spermatozoa with swollen tail ('g' and 'b' forms) was also assessed.

21 of 41 study samples were kept in electrolyte-free glucose and fructose solutions, and 8 samples – in all three preservatives. Results are given as mean ± SE. Wilcoxon's matched pairs test was used to calculate significant differences in motility and swollen sperm content following storage in glucose and fructose (N = 21) and in all three preservatives (N = 8). Statistic calculations and graphics were performed with software STATISTICA 5.0 (StatSoft, Inc.).

3. Results

20 of 41 sperm samples were preserved only in glucose solution, 21 were stored both in glucose and fructose preservatives and 8 samples were stored in all three solutions including sorbitol.

Motility of spermatozoa recovered in each sample after fortnight cool storage in all three electrolyte-free solutions followed by revitalization within 2 hours. Significant differences in motility for all three preservatives were revealed ($p<0.05$). The percentage of motile spermatozoa with normal tail morphology was 58.8 ± 3.1% (N = 41), 13.3 ± 2.3% (N = 8) and 44.5 ± 4.5% (N = 21) for glucose, sorbitol and fructose respectively ([Fig.1] Fig.1). Interestingly, that one of the cool preserved samples was donor sperm stored before at cryobank for almost 3 years. Despite this, 76% (glucose solution)

and 54% (fructose solution) of these spermatozoa recovered motility.

In our study hypo-osmotic swelling was not detected in all electrolyte-free solutions neither before nor after storage until preservatives were removed and replaced with incubation medium. The rate of swollen sperm in glucose, sorbitol and fructose samples was 5.3 ± 0.6% (N = 41), 66.5 ± 2.6% (N = 8), 14.1 ± 3.4% (N = 21) respectively ([Fig.2] Fig.2); these differences in percentages were also significant ($p<0.05$).

4. Conclusion

Human sperm recover motility while stored for at least two weeks without freezing at $+4^{o}C$ in electrolyte-free solutions of glucose, fructose and sorbitol. The highest rate of sperm preservation was found in electrolyte-free solution of glucose, followed by fructose solution, with sorbitol solution showing the lowest rate of preservation.

Genetic safety of cool sperm preservation in electrolyte-free glucose solution shown by Riel et al. (2008) together with high efficiency and technical simplicity of such approach also demonstrated in our study provide new possibilities for ART.

5. References

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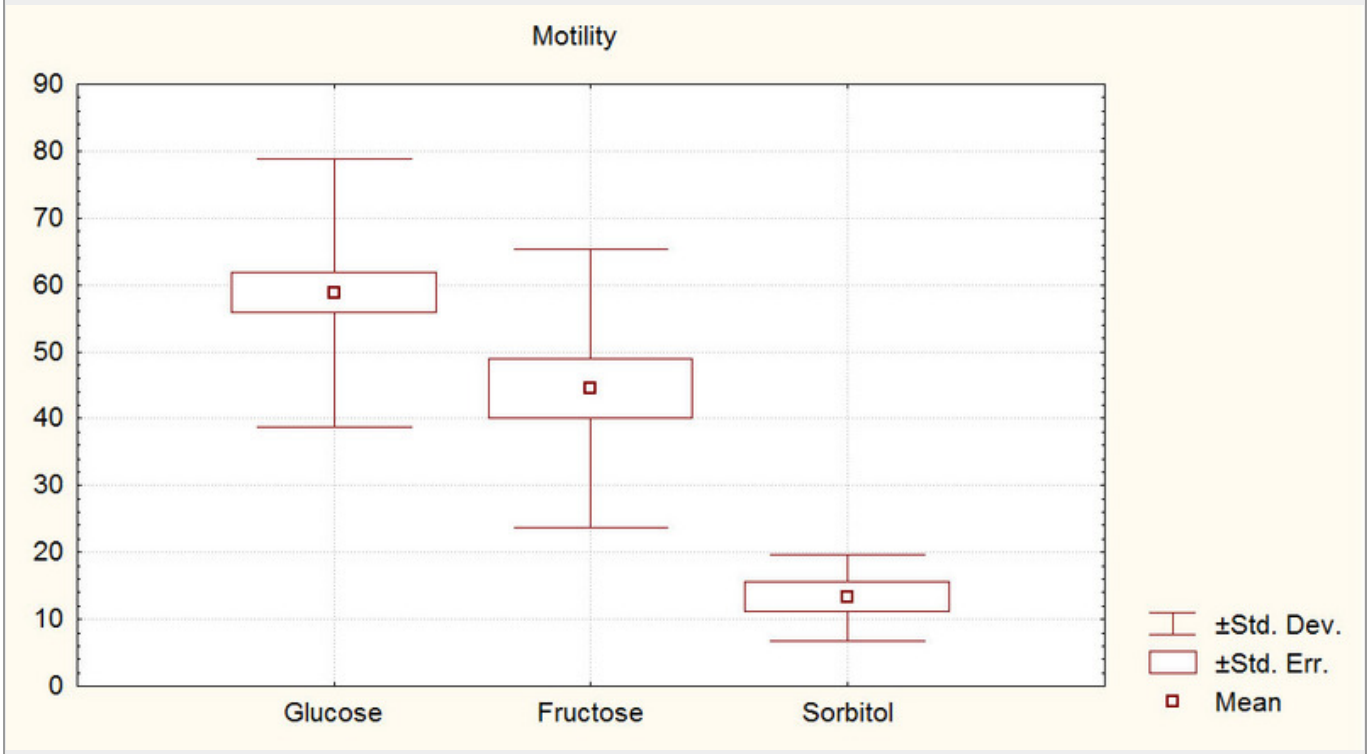
Kanno H., Saito K., Ogawa T. et al. Viability and function of human sperm in electrolyte-free cold preservation. Fertil Steril 1998; 69: 127-131.

Frenette G., Thabet M., Sullivan R. Polyol pathway in human epididymis and semen. J Androl 2006; 27: 233-239.

Riel J.M., Huang T.F. and Ward M. A. Freezing-free preservation of human spermatozoa – a pilot study. Archives of Andrology 2007; 53: 275-284.

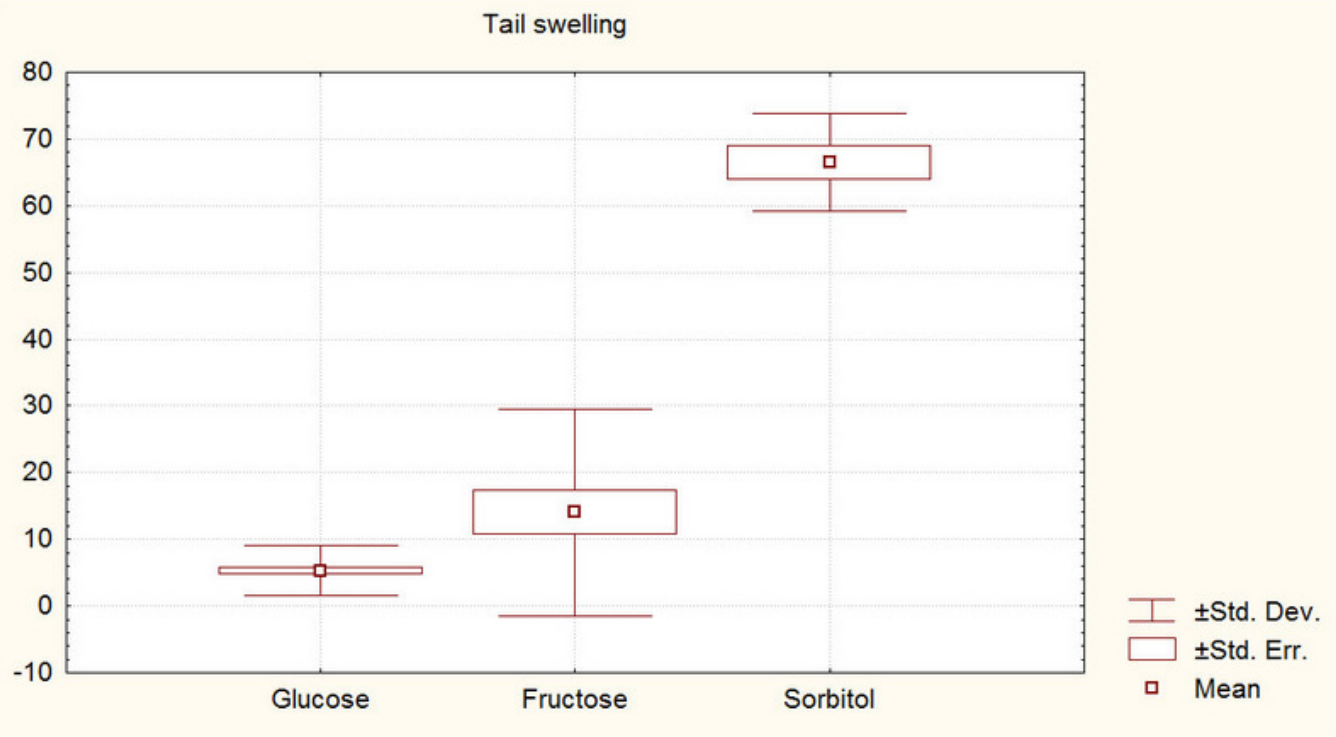
6. Mediafiles

Fig.1



Sperm motility after fortnight storage without freezing

Fig.2



Tail swelling rate after storage and subsequent revitalization