

CRYOPRESERVATION OF A SMALL NUMBER OF HUMAN SPERM USING SODIUM ALGINATE MATRIX

Rika Suzuki-Migishima¹, Kanako Ohsumi¹, Shu Hashimoto¹, Tomoko Inoue¹, Fujio Migishima¹, Keijiro Ito¹, Hirotsugu Oku¹, Yoshiharu Morimoto¹

¹ IVF Namba Clinic (Japan)

Purpose: Cryopreservation of a small number of spermatozoa is important in oligozoospermia and azoospermia patients. Sodium alginate is produced by brown algae, and not harmful to cells. Cryopreservation of sperm in alginate gel has been investigated in mice, bovine and human, but the procedure for small number of human sperm has not yet been established. In this study, we investigated freezing conditions using alginate gel for a small number of human sperm.

Materials and Methods: We used ejaculated semen from infertile men who gave informed consent. We assessed time required for complete liquefaction of 3 µl of 1% alginate gel in various concentration of alginate lyase, effect of alginate lyase on sperm motility, and volume and concentration of sodium alginate. A few number of sperm in droplet diluted with KSII: HTF (1:1) were put into 3 or 5 µl of 0.5% or 1% sodium alginate using micro-manipulator under reverse microscope (x400). Sodium alginate containing sperm gelled with immersion in Ca²⁺ solution and the gels were transferred to freezing solution, KSII: HTF (1:1), in tubes. It was left for 10 minutes at room temperature (RT), was kept for 10 minutes on vapor of nitrogen liquid (LN₂), and was inserted into LN₂. The tubes were thawed at RT until the solutions were completely thawed. Alginate gels were transferred to 5µl of 40 unit/ml alginate lyase solution and liquefied at 37 C for 10 minutes. Retrieved sperms were counted under reverse microscope (x400).

Results: Time required for liquefaction of 3 µl of 1% alginate gel in 10unit/ml alginate lyase (60 min) was longer than those in 20, 40 and 80 units/ml (5 min). The concentration of alginate lyase (10, 20, 40 and 80 unit/ml) did not affect the sperm motility after 2h treatment. There were no differences in recovery rates between 3 and 5µl of 0.5% alginate gel (58% and 58%, P>0.05) and between 3 and 5µl of 1% gel (90% and 77%, P>0.05). The recovery rate with 1% gel was significantly higher than that of 0.5% gel using 3µl gel (58% vs. 90% P<0.05). Some sperm might be lost assumably due to fragility of 0.5% alginate gel compared with 1 % gel. In addition, 5 µl of 1% gel might be large to liquefy in small volume of alginate lyase solution. As a result, highest recovery rate was obtained with 3 µl of 1% gel.

Conclusion: It was suggested that alginate gel was useful for cryopreservation of a small number of human sperm.