Ovarian cryopreservation

The whole ovaries were placed in 1.2ml-cryovials (Sigma-Aldrich®, Inc.) in M2 medium plus HEPES without Penicilin and Streptomycin (M2-Sigma-Aldrich®, Inc.) and with 1.4M dimethyl sulfoxide (DMSO) (Sigma-Aldrich®, Inc.) as a cryoprotector and were held at room temperature for 5 min. The cryovials were sealed by twisting their caps and were placed in a temperature-programmed freezer (CL-8800, Cryogenesis software, Freezer Control) and cooled from 25^{o}C to 10^{o}C at a rate of 1^{o}C/min, then to -7^{o}C at a rate of 0.5^{o}C/min, and maintained at -7^{o}C for 5 min. Then, ice nucleation was manually induced using pre-cooled forceps, and the temperature was held at -7^{o}C for another 5 min to allow for the release of latent heat fusion. The tissue was then frozen at -55^{o}C at a rate of 0,5^{o}C/min, plunged into liquid nitrogen at -196^{o}C and stored for 24 hours.

For thawing, cryovials were removed from liquid nitrogen and held at room temperature until the ice melted. The ovaries were washed two times for 5 min in fresh M2 medium, then shaken gently to remove the cryoprotectant before further processing. The ovaries were maintained in M2 at room temperature until the transplant.