

## National Physicians Cooperative of the Oncofertility Consortium<sup>®</sup>

### Section 3. Slow Cooling for Ovarian Tissue: Setting up your programmable freezer

#### Background:

1. Ovarian tissue is cryopreserved using a slow cooling technique and requires a programmable freezer that permits manual seeding.
2. Ovarian tissue is frozen in **vials** so your freezer must accommodate vials.
3. When a whole ovary is frozen, there will be approximately 30 vials to freeze.
4. Your freezer must accommodate a large number of vials in a single freezing run.
5. We recommend that you use a labeling machine to label vials to reduce errors.

#### Ramps for the Freezing Run:

1. Please program your freezing in advance for ovarian tissue freezing.
2. Please follow these ramps exactly, please do not make changes.
  - Load vials in to programmable freezer at 5.0°C
  - Cool at a rate of -2.0°C/minute to -7°C
  - Hold for 10 minutes and then **manually seed** all of the vials
  - Hold for an additional 10 minutes for ice crystal formation
  - Cool at a rate of -0.3°C/minute to -30°C
  - Plunge into liquid nitrogen

#### Technical Notes:

1. We recommend programming your freezer to
  - Alarm when it is time to seed.
  - Stay at the seed temperature until you prompt the machine to continue once the seeding is complete (i.e. the program should not continue on automatically in case the seeding has not occurred or has not been completed yet).
2. Freezer should be plugged into backup power source in case of power failure during the run.
3. You should print a chart of the freezing run for quality control purposes.
4. If you are running the freezing machine off a laptop computer, ensure that the computer's "sleep" function is disabled.