Preparation of Semen Specimens for Cryopreservation Morning or Afternoon Specimen Collection, Washed Versus Unwashed. Which is better?

Centola, G.M., and Walter, D.D.
Manhattan Cryobank, New York, New York

Objective: To determine differences in semen parameters between morning or afternoon collection, response to cryopreservative addition and washed versus unwashed specimens.

Design: Retrospective analysis of sperm bank database between 2014 and 2018.

Materials and Methods: Of 1179 specimens from 22 donors, 720 were collected before noon, 458 after noon. 252 ejaculates were cryopreserved as neat, unwashed semen, while 932 specimens were gradient prepared prior to cryopreservation. A standard semen analysis was performed 30 minutes after ejaculation. Raw semen was diluted 1:1 with TEST-yolk buffer (Irvine Scientific), placed in vials, and frozen in liquid nitrogen vapor prior to storage in the liquid phase. For gradient preparation, raw semen was layered onto an 80% layer followed by centrifugation at 320g for 20 minutes. The pellet was washed, resuspended in HTF, followed by addition of the cryopreservative and frozen as above. Following a minimum of 48 hours, the test vials were thawed, and the post thaw count and % motility were determined. Data was analyzed using a 2-tailed, unpaired t-test with significance set at $p \leq 0.05$.

Results: When all donor data was compared, there was no statistical difference between morning and afternoon semen collection for volume (p=0.006), count (p=0.49), motile count (p=0.59) and % motility (p = 0.95). When AM to PM collections were compared for each donor, no statistical difference was seen for all parameters in only two donors. Volume was significantly higher in 5 donors; count was higher in AM for 2 donors and in PM for 2 donors; % motility higher for 1 donor in AM and in PM for 1 other donor. The gradient prepared specimens showed significantly (P= 0.0001) higher post-thaw motility and cryosurvival rate than unwashed specimens.

Conclusions: There appears to be no difference in semen specimens collected in the morning versus in the afternoon, although there was a trend for higher semen volume in the afternoon specimens. There were differences in individuals between morning and afternoon collections which should be considered when evaluating sperm donors as well as clients, particularly for use in ART procedures. Gradient preparation prior to freezing results in better motility, motile count and cryosurvival before freezing and after thaw, suggesting that this method is more favorable for donor sperm cryopreservation.

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