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Practical Considerations in the Preparation of Sperm Prior to Cryopreservation

Androlog Summary

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Note: Postings to *Androlog* have been lightly edited before publication.

Clinicians continue to develop a broader understanding of the utility of cryopreserving sperm in a variety of settings. Such settings range from the preservation of sperm before initiation of chemotherapy or radiation therapy in a man who desires future fertility to the harvesting of sperm during vasectomy reversal to allow ongoing fertility options in the event of a failed reconstruction.

Given this interest in sperm cryopreservation, andrologists are understandably seeking the best techniques for effective sperm cryopreservation. In this edition of *From Androlog,* Grace Centola inquires regarding the best method for washing sperm before cryopreservation.

The discussion began when Grace Centola posted this timely inquiry to *Androlog:*

> I am trying to get a good technique for washing sperm before cryopreservation. We freeze andrology patients' sperm for many reasons prior to use with intrauterine insemination (IUI). Specimens that are frozen as raw semen and then washed upon thawing really don't always do that well, even with a simple centrifugation wash. Any input from *Androlog* members will be much appreciated.
>
> For example, should the prefreeze wash be by a 1-layer gradient? What cryopreservative to use and what ratio? If raw semen is thawed, how should it be prepared for IUI?

Suresh Sikka made the following recommendations:

> Semen dilution (1:5 [vol/vol]) in sperm wash media, followed by single centrifugation, resuspension, and use of certain cryoprotectants before freezing of these washed sperm in TEST-yolk buffer (TYB), improves postthaw recovery. Multiple centrifugation steps (eg, by using density gradient media) should be avoided.
>
> Refer to our earlier publications: 1) Bell, Wang R, Hellstrom WJ, Sikka SC. Effect of cryopreservation protocol on sperm membrane LPO and recovery of motile human sperm. *J Androl.* 1993;14:472–478; and 2) Wang, Sikka SC, Veeraragavan K, Bell M, Hellstrom WJ. PAF and PTX as human sperm cryoprotectants. *Fertil Steril.* 1993;60: 711–715.

Sam Henrichs also responded, providing additional suggestions, which were based on his own experience:

> I've always had good luck in spinning the raw frozen/thawed specimens against an 80%–90% Percoll/Puresperm/Isolate or similar product. I like to have the sperm and Puresperm at body temperature. To optimize the motility of the sperm (vs using room temperature), I'll first put up to 1 mL of thawed semen on top of 1 mL of the 80% Puresperm, spin at 300 × *g* for 15 minutes, and then combine the 80% fractions and clean up by diluting with whatever I'm using for my IUI media. I spin for as short a period and as simply as possible to get a complete pellet, such as at 2 to 300 × *g* for 5 to 10 minutes. I try never to use in excess of 300 × *g* when working with the frozen-thawed cells. Of course, with poorer-quality specimens, I may use multiple specimens to produce one nice-looking IUI specimen. When using 2 or more units of donor sperm this way, you can end up with an IUI specimen that looks much nicer than many washed fresh specimens.

Stan Grossman offered some additional ''laboratory pearls'' from his andrology facility:

> If you wish to process semen prefreeze, I would suggest a 2-layer (45%/90%) ''mini'' gradient wash with your choice of commercial media, such as Enhance-S-Plus from Conception Technologies. At our center, we have successfully used freezing medium available from I.S. as the cryopreservant.
>
> Thawed raw semen should be softly washed before IUI. Again, we rely on I.S. for their sperm washing medium for this process.

Kerem Dirican rounded out this very informative set of suggestions with some helpful hints from his experience in this area:

> Reply to washing sperm before cryopreservation from Grace Centola. My professional experience is to perform a 1-step simple wash before applying TYB (but don't ever use a selection procedure like spermgrad or swim-up before freezing), and adjust the sample to a desired volume. In my opinion, the most critical time is the thawing. If the sample is good enough to perform swim-up, it is very important to add the sperm wash media very slowly in order not to damage the cells by an osmotic shock. If the sample requires a density gradient centrifugation (DGC), I prefer not to perform a wash step before applying DGC. I place the thawed sample directly on the gradient for 20 minutes at 200 to 600 × *g* (according to the quality of the sample) and wash twice after the DGC (1800 × *g* and 4 mL of wash media for each step). I think this procedure results in minimum osmotic shock to the cells. Best regards, Kerem Dirican

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