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Semen freezing information

The semen freezing protocol we follow at Reproductive Revolutions is different from most freezing facilities. We perform extensive testing both pre- and post thaw, to ensure that the semen is of adequate quality to freeze, and to ensure the quality of the semen post thaw provides an adequate, normal, motile sperm in each breeding dose to be able to expect normal fertility. In some cases, we may need to adjust the number of straws in a breeding dose after post thaw analysis, to provide a quality product to the bitch owner. There is, of course, no way to guarantee fertility, but we do everything we can to ensure that each bitch owner has a good chance to get the bitch pregnant when using semen that we have frozen.

We do a complete semen evaluation prior to freezing, which includes a sperm motility assessment where the sample is diluted properly to assess individual sperm motility. We assess both total and progressive sperm motility and the velocity of sperm movement. We perform a sperm count using a nucleocounter, a machine which only counts sperm – it is not confused by other cellular debris - which makes the count more accurate. We perform a stained morphology slide using eoisin-nigrosin staining, which not only allows us to assess cellular morphology but also sperm viability by differentiating live from dead sperm via uptake of stain across the non-viable membrane of the dead sperm.

We look for all types of sperm defects that may affect fertility. If there is a poor chance of getting a quality sample based on semen quality, we will recommend discarding the sample and not spending the money on the freeze. This is in the best interest of the dog owner and the dog's reputations and in the best interest of the bitch owner who will use the semen in the future. If we have concerns about the semen quality it is included in the freezing report, so this information is available to the bitch owner before using the semen, allowing them to make the best decision with their reproductive veterinarian as to whether to use the semen and if they choose to use it, how to best manage their bitch. Without accurate ovulation timing, even the best semen may not result in pregnancy.

We freeze in Uppsala extender unless previous freezes dictate using a different extender. This extender has amazing longevity, with many samples remaining 30% or higher in motility, at room temperature, at 24 or more hours after the semen is thawed. This extender was created by Dr. Catharina Linde Forsberg of Sweden, who is generous enough to share her recipe with the world.

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After freezing, we allow the sperm to remain frozen for at least 24 hours prior to post-thaw analysis to ensure our post thaw results are accurate. Thawing immediately after the freeze is performed may result in falsely high post thaw motilities. Our post thaw analysis includes assessing both total and progressive motility, longevity, viability, acrosome status and membrane integrity. Most semen freezing facilities only assess motility after the semen is thawed.

Viability is assessed using the nucleocounter which is a flow cytometer and stains the normal DNA in the sperm head a different color than damaged DNA. So the percent of sperm that have normal DNA integrity are differentiated from those that may have been damaged by the freeze. Longevity is assessed by leaving sperm at room temp for 24 hours and then reassessing motility over time.

The acrosome is assessed by staining with a special stain called Spermac which specifically stains for the acrosome and allows for identification of the presence of the acrosomal cap, which is the structure on the sperm head that contains the enzymes necessary to penetrate the egg. In some dogs, this may be damaged by freezing, rendering these sperm unable to fertilize the eggs despite their remaining motile. Membrane integrity is assessed using the hypoosmotic swelling test (HOST) and this test tells us whether the sperm membrane is still functional after freezing or if it was damaged during the freezing process.

We do not provide long term storage here. Most of our clients will store their sermen at Zoetis, in Kansas City, MO. Zoetis is a world class storage facility. They have state of the art security systems and protocols in place to protect the semen that is stored there. They are also capable of assisting us with any international semen shipments we have. It costs \$103/year/dog, to store semen, regardless of the number of doses stored. There is no charge to get the semen to their storage facility. If the semen ships back to another Zoetis freeze center in the continental US (we are one here), the cost is a flat rate \$100 fee. There is no charge for tank rental, semen prep, or Fedex shipment. If the semen ships to a location that is not a Zoetis freeze center or to international locations (including Zoetis centers outside the continental US), standard shipping charges apply which usually runs about \$800-1500, depending on where the semen is being shipped.

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Pricing:

Our costs for freezing are as follows:

First visit \$700 for up to 4 doses semen; \$70/dose above and beyond 4.

Repeat visits \$600 for up to 4 doses semen; \$70/dose above and beyond 4.

Brucellosis \$85.50 –always performed on the first visit and then every 2 months or longer thereafter (that means if you do more than 1 collection in less than 2 months you don't need another test but over 2 months between collections, you do)

Dogs registered to the American Kennel Club (AKC) must have a DNA parentage test done. The kit for this can be ordered on the AKC website and the client takes the swab and sends it in directly or we can take the swab (for a fee of \$20.50) at the time of the freeze and complete the paperwork for you. Then we give the owner the swab to mail in to AKC after including payment of \$50 for the test.

Microchip – all dogs having semen frozen need permanent ID – this is either a microchip or a readable tattoo. If your dog doesn't have a microchip, we can place one at the time of the collection for \$46.20.

Teaser – we strongly recommend use of a teaser bitch (bitch in proestrus or estrus) for all freezes. This will always provide a better sample because the male is more stimulated during the collection, which means more sperm ejaculated. If the client doesn't have a teaser, we can arrange for one for \$50/visit. This money is credited to the bitch owner who takes time out of their day to help us out and bring their bitch in to assist with collection.

Semen that is being shipped internationally may require further testing either before collection, at the time of collection or a specific time frame after collection, depending on the country where it is being shipped. Please be sure to mention to us when you call to schedule, that the semen is going internationally so we can check on testing requirements. In most cases, an import permit is required by the bitch owner as well.

The number of doses in a collection will depend on the dog's age, semen quality, number of sperm ejaculated, the quality of the freeze and the absence or presence of prostatic disease (the latter of which may result in poor post thaw quality). We do not freeze dogs with overt signs of prostate disease at the time of collection (blood in the ejaculate) but sometimes, prostate disease is not evident at collection but rather is suspected when post thaw analysis is not what is expected. Dogs with prostate disease, should have their prostate disease treated before freezing.

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On average, toy and small dogs produce 1-2 doses per freeze, medium dogs produce 3-4 doses, large dogs produce 4-6 doses, and giant breed dogs produce 6-8 doses. There are exceptions to these numbers in both directions, but these are averages. We try to package breeding doses of 100 million motile sperm for toy and small breeds dogs; 100-150 million for medium breeds; 150-200 million for large breeds; and 200-250 million for giant breeds. With these doses, the bitch owner can plan on using 1 breeding unit per cycle.

Please feel free to call us with other questions regarding semen freezing.

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