

# The Semen Looked Bad - Or Is It?

By Jos Mottershead and Kathy St. Martin

During the course of the breeding season - especially on the *Equine-Reproduction.com* bulletin board or the EquineRepro@yahoogroups.com e-mail list - we frequently hear the groan "the shipped semen we received looked terrible - it only had 10% progressive motility - so we don't think Old Bessie will get pregnant". The percentage and the mare's name may vary, but the focus remains the same - the low percentage progressive motility of the sperm. The only problem is that - to a point - low progressive motility doesn't really mean anything. When the mare does get pregnant, the mare owner is astounded and impressed with the quality of the mare and her fertility.

What then should we look at when we receive semen, and what does constitute "poor quality" worthy of concern?

The first important point to consider is that it is not the *percentage* of progressively motile sperm in the ejaculate that counts, but rather the total number of progressively motile and morphologically normal sperm (PMMNS)! In other words - within reason - people receiving cooled semen shipments would do well to lose their fixation with percentages, and focus on total numbers instead.

For many years the "standard" insemination dose has been considered to consist of 500 million PMMNS. The research that arrived at this number was performed primarily at Colorado State University in the 1970's but with newer breeding methods and a better understanding of reproductive issues, it is now clear that the research and those figures are somewhat outdated and likely erroneous. For many years, the lower end number - which was probably of more importance in many cases than the upper end number - was not evaluated, or was ignored, because there was an obsession with the  $500 \times 10^6$  (five hundred million) number. At *Equine-Reproduction.com* we had long felt that provided the insemination was performed in close proximity to ovulation - ideally inseminate in the afternoon and have the mare ovulate that evening or night - the number below which one did start to see a reduction in pregnancy rates was  $100 \times 10^6$  (100 million) if using standard insemination methods. Recent research has supported our hypothesis <sup>1</sup> and indicates that a suitable insemination dose is comprised of between 100-500 million PMMNS. The same CSU publication that indicated the "optimal" insemination dose as being  $500 \times 10^6$  PMMNS indicated that insemination doses containing in excess of that number were not shown to significantly increase pregnancy rates. This "upper end number" has been supported in clinical settings as being accurate.

To achieve the supposed "standard" insemination dose of 500 million PMMNS one typically ships a minimum of 1 billion ( $100 \times 10^9$ ) sperm, anticipating a die-off rate of 50% (i.e.  $50\% \times 1 \text{ billion} = 500 \text{ million PMMNS}$  at the time of insemination). If however, one has as low as 10% PMMNS at the time of insemination, there is still an insemination dose of  $100 \times 10^9$  PMMNS available, which the new research indicates as being adequate, and with a suitably timed ovulation there will be no reduction in pregnancy rates. Hence even though there is supposedly low progressive motility, pregnancy rates are not affected.

## How should one evaluate cooled transported semen upon receipt?

1. Gently mix the semen to ensure resuspension of sperm that may have settled to the bottom of the container during transit;
2. Take a small sample of the semen, place it on a warm microscope slide and continue to warm it to body temperature for about 5 minutes (this can be achieved by using the in-the field slide warmer described elsewhere on *Equine-Reproduction.com*);
3. View and evaluate multiple fields under the microscope - do not look at a single field and then contemplate suicide! Fields may vary in how they warmed. If you find all sperm are dead, or there is very poor motility, prepare another slide and re-evaluate. You may have inadvertently introduced a spermicidal contaminant or accidentally "cooked" the sample while warming it. If that happens, ensure an absence of contamination, make sure your slide warmer is not too hot, and reduce the time you warm the semen;
4. Make sure there are not too many sperm on the slide - you cannot get an accurate determination if there are too many sperm in the field of view. If you find there are too many, prepare a second sample with a smaller drop of semen. Ideally there will be about 20-60 sperm visible under the 400x power view;

5. Make sure that you differentiate between *overall* and *progressive* motility - it is progressive motility that you are most interested in;
6. Once the progressive motility has been determined, refer to the paperwork that should have accompanied the shipment to determine the number of sperm that have been put in each insemination dose. Multiply the percentage PMMNS by the number of sperm to obtain the number of PMMNS present in the insemination dose - you want greater than 100 million to be the resulting figure;
7. If there is no paperwork showing the number of sperm present (the paperwork should also show other salient information such as the stallion's name, and the type of antibiotic present in the extender) be aware that this may indicate that suitable preparation work is not being performed at the shipping end! **There is no excuse for not including paperwork that gives accurate information!!!!**
8. If there are fewer than 100 million PMMNS present in a single insemination dose, it may be desirable to inseminate both doses at the same time (provided two doses were shipped). Note that this is not normally recommended, but in these circumstances it may be beneficial.
9. Evaluate and record other aspects of the semen quality:
  - If the shipment has a grey colouration - rather than a cream or white colour - that may indicate inadequate washing of the stallion's penis prior to collection;
  - There should not be gel present in the semen. Both gel and pieces of dirt, smegma, skin or other detritus should have been filtered out immediately after collection;
  - A pink or yellow tint to the semen may indicate the presence of blood or urine, both of which are likely to render reduced or no fertility (and should have been observed at the time of collection and semen preparation);
  - The final sperm concentration should be between 25-50 million sperm/ml. If the concentration is outside these parameters, that may indicate over- or under-dilution with semen extender, which can have a negative impact on longevity and viability of the sperm.

## Then what?

It may be worth using oxytocin to assist in clearing excess fluids from the uterus. This may be especially beneficial if, as a result of low viable sperm numbers, both insemination doses of semen are inseminated at the same time, or if one is dealing with a mare with delayed uterine clearance issues. Following an oxytocin protocol such as the one outlined elsewhere on the *Equine-Reproduction.com* web site may be beneficial.

In the event that the semen quality is obviously poor, one can perform further evaluations and keep a record for future use. If the semen concentration looks incorrect (it should, if extended correctly, be between 25 and 50 million per ml), the actual concentration can be established by counting the sperm using a hemacytometer. Many people think that once the semen is extended it cannot be counted, but this is incorrect. Once the concentration has been determined, multiplying that number by the total volume in ml's will give one the total number of sperm that were shipped - remember this is generally in excess of 1 billion sperm, anticipating a die-off rate of 50% by the time of insemination. Finally, a video eyepiece can be purchased fairly economically that fits in the eyepiece of your microscope and connects to a VCR or your computer. This will allow a video record to be made of the actual motility and condition of the semen. At *Equine-Reproduction.com* we evaluate warmed motility and do a concentration (and total number) count as a matter of routine on all semen shipments that we receive. We will also video samples that we feel are sub-standard.

Don't forget to confirm ovulation! Mares do not always follow the rules, and may delay ovulation past the anticipated time (or of course ovulate sooner than one thinks they should!). Confirming ovulation is a cheap insurance policy to make sure that you don't need a second shipment of semen.

Then finally, possibly the most important point - cross your fingers, hope for the best, and check by ultrasound 14-15 days after the mare ovulated!

---

## Summary:

### Observation:

**It is clear that many breeders using transported semen need to lose their obsession with**

## progressive (or rather the lack of progressive) motility!

### Conclusion:

- A better understanding by breeders of the significance of the number of PMMNS is needed;
- Evaluation should be made of a suitably warmed aliquot of the semen to determine progressive motility;
- During evaluation reference to paperwork (that should be included in well-prepared shipments) needs to be made to determine the number of PMMNS present;
- Only if the PMMNS number is seen to be below 100 million need the mare owner be concerned;
- Other evaluations may be made for permanent record in the event that the quality of the shipment is found to be genuinely lacking.

---

### References

**1: Brinsko S.;** (2006) Insemination doses: How low can we go? *Theriogenology* 66:3 543:550 (Proc. Annual Conference of the Society for Theriogenology).

---

### Glossary of Terms

100 x 10 <sup>6</sup>	This is the mathematical abbreviation indicating the initial number <i>multiplied by 10 to the power of the superscript number</i> , so in this particular instance it is 100 to the power of 6, or 100 to the power of 10 x 10 x 10 x 10 x 10, or in other words 1 million. One <i>billion</i> is written 100 x 10 <sup>9</sup> (at least in North America - there are some variances in other parts of the world).
PMMNS	Progressively motile, morphologically normal sperm
Progressive motility	Progressively motility refers to sperm that are moving forwards in a [close to] straight line. This differentiates from sperm that are merely <i>motile</i> , which are moving but may be traveling backwards, sideways or in circles rather than solely forwards.

---

© 2007 Equine-Reproduction.com

Use of article permitted only upon receipt of required permission and with necessary accreditation.  
Please contact us for further details of article use requirements. Other conditions may apply.