

**THE LATEST FROZEN SEMEN TREND:  
ARE ONCE-DAILY EXAMINATIONS REALLY THE FUTURE?  
-Another Perspective-**

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This past fall, I attended the West Coast Equine Reproduction Symposium in Solvang, California. A very nice, well-rounded series of lectures were presented dealing with various topics of the stallion, the cycling and pregnant mare, and the newborn foal. The topic that seemed to take front stage, at least in my mind, was the presentation of data promoting the use of 2 frozen semen insemination doses per cycle while only evaluating follicle development once per day. For years, it was thought that optimal results with frozen semen would only be achieved if the developing preovulatory follicle was examined every 6 to 8 hours so insemination can occur as close to ovulation as possible. Recent work by Dr. Barbacini, however, seemed to dispel that thought. A clinical trial was performed in Italy during the 2000 and 2001 breeding seasons. Mares were either examined 4-times per day and inseminated once within 6 hours after ovulation or examined for follicle activity once daily with inseminations occurring at 24 and 40 hours after hCG administration. Pregnancy rates were found to be similar between the 2 groups of mares, yielding 71% and 76% pregnancy rate per cycle, respectively. Exceptionally high pregnancy rates were seen in both groups because the frozen semen used was chosen for its already proven fertility.

Upon evaluating the data, it made sense that once daily examinations were sufficient because ovulations were occurring in a predictable manner. By using an agent such as hCG or Ovuplant to induce ovulation, inseminations can be strategically timed so that the majority of mares will be bred within 12 hours before to 6 hours after ovulation with at least 1 dose of semen. The number of examinations performed becomes irrelevant at that time. Could this mean the end of late night/early morning examinations? Could

frozen semen management really be part of a 9 to 5 day? It seems too good to be true. And whenever I get that feeling, it usually puts a bit of skepticism in my mind. I remember 2 years ago when everyone jumped on the bandwagon to use Ovuplant, instead of hCG. It was only after its widespread use did we notice some of the negative side effects this synthetic hormone had on the estrous cycle.

So for the purpose of this article, I am going to play devil's advocate. Far be it from me to quash a seemingly great management scheme but I do want to point out a few circumstances that may make one wonder if this strategy is appropriate. First of all, the proposed plan for reproductive management only works if the mare is given and responds appropriately to an ovulatory agent. The veterinarian monitoring the mare still needs to be comfortable with knowing when to administer such agents. Giving hCG or Ovuplant before the developing follicle is mature enough to respond will only result in a failure to ovulate when predicted. The consequence may be inseminating one or both doses too early, thus wasting valuable semen. Of course, if ovulatory agents are given too late in the cycle then ovulation might spontaneously occur before its anticipated time. In this scenario, inseminations will occur after ovulation and quite possibly, beyond the 6-hour window of opportunity. Induction of ovulation should only occur when the mare is in heat, has prominent endometrial edema and a mature 35-, 40- or in some cases, 45-mm follicle present. Even using these criteria, there will be a certain percentage of mares that show variability in response to treatment. According to Duchamp and associates (1987), close to 84% of the mares should ovulate within 48 hours once given 2000-2500 iu of hCG. Ovuplant has been shown to be equally effective (83-90%) in

inducing ovulations (Farquhar et al., 2000). McKinnon and coworkers (1997) reported that the average time to ovulation was shorter when hCG was administered ( $36 \pm 4$  hrs) versus Ovuplant ( $41 \pm 3$  hrs).

One advantage to multiple-daily versus once-daily examinations is that inappropriately-timed inseminations are minimized or eliminated. Frequent palpation/ultrasound exams allow one to closely assess remodeling of the follicle. Changes in softness, thickening of the follicle wall, shape, and presence of granulosa cells within the follicular fluid are all parameters that can be monitored. One may want to delay breeding if the follicle isn't maturing as anticipated. If a mare ovulates before expected, there's only a 6-hour window that it could have occurred in. Immediate insemination can be performed with presumed no affect on pregnancy rates. You don't have that luxury when examining once every 24 hours.

Quite often, breeding protocols require using only 1 dose per cycle. Under those circumstances, follicle assessment needs to occur every 6 hours so the insemination is done immediately after ovulation. A single exam every 24 hours would simply be inadequate. A complaint one might have with this new management scheme is that 2 doses of semen are utilized when only 1 dose per cycle is necessary. Isn't expensive semen being wasted? An interesting study was recently done in Colorado whereby 40 mares were inseminated in one of two manners: Group 1 was inseminated with 400 million total spermatozoa at 24 hours and again at 40 hours after hCG treatment. Mares in Group 2 received one insemination of 800 million total spermatozoa within 6 hours post-ovulation. Pregnancy rates were similar between the 2 groups (55 and 60%, respectively). These results lead one to believe that no additional sperm are needed for the proposed, 2 dose scheme versus a single, post-ovulatory breeding. In 1998, Leipold and coworkers reported no improvement in pregnancy rates when mares were bred with more than twice the number of progressively motile sperm normally used in traditional breeding doses (800 million vs. 320 million pms). Although not confirmed, it is believed that this study compared

pregnancy rates when all the semen was inseminated at the same time. In contrast, there is some evidence to suggest that inseminating with at least 2 doses, at different times, does increase the chances of getting a pregnancy. Palmer and Magistrini (1992) found an improvement in pregnancy rates when mares were inseminated with 2 or more doses compared to a single dose per cycle. This was confirmed by Metcalf (2000) whereby 11 mares were inseminated just prior to ovulation with frozen semen from Stallion A. Six to 10 hours after ovulation, mares were inseminated a second time with frozen semen from Stallion B. These stallions were selected because of their fertile semen. Interestingly, 9 of 11 mares were pregnant. Four mares conceived from Stallion A and 5 mares were pregnant from Stallion B.

Researchers often will use proven semen for fertility trials in order to eliminate variables that may alter the outcome of the study. However, by using only "the best" semen, results obtained may not be representative of a general population of frozen semen. With this in mind, the question was raised at the Symposium as to whether semen from certain stallions could have a better chance of obtaining pregnancies if mares were monitored 4-times daily versus once daily. Dr. Barbacini candidly stated his belief that a closer assessment of ovulation may indeed be appropriate in some instances. At ERC, we speculate that frozen semen from some individuals has a shorter lifespan than others and therefore, inseminations as close as 12 hours prior to ovulation could be insufficient in these cases. Undoubtedly, some frozen semen could be inseminated as much as 24 hours before ovulation and still have adequate pregnancy rates. This tends to be however, a very stallion-dependent phenomenon.

It is well known that endometritis, or inflammation of the uterine lining, occurs following all inseminations. However, the physiological reaction is more prolonged when using frozen semen. In some mares, within only a few hours after semen exposure there may be an accumulation of uterine fluid. This develops as part of the inflammatory process. If one is concerned about the fluid, then either oxytocin can

be administered and/or a uterine lavage performed as early as 6 to 8 hours after insemination. Removing the irritant expeditiously will prevent the uterine environment from becoming too harsh for the anticipated conceptus. An advantage to assessing follicle growth multiple times per day is that it also allows for repeated evaluations of uterine fluid build up. Proper management of fluid may be the deciding factor on whether or not the mare is diagnosed in foal.

The recent data from Italy has made us reevaluate our present breeding protocol with frozen semen. It certainly is enticing to think that

we could go to a once-daily examination schedule without affecting pregnancy rates. The fact remains however that there are certain circumstances whereby monitoring follicle activity more than once per day is still necessary. Therefore at this time, our policy at ERC will more than likely utilize both management schemes. Our goal is to do what's best for the individual breeding, not just what's most convenient. Below are a set of guidelines by which we will operate the frozen semen breeding program this coming season. Of course, there may be exceptions to the rules on occasion.

<b>CATEGORY</b>	<b>EXAMINATION SCHEDULE</b>
Reproductively sound mares bred with fertile semen	1 time per day
Reproductively sound mares bred with semen of unknown fertility	4 times per day after hCG administration
Reproductively sound mares bred with a single dose	4 times per day after hCG administration
Mares with irregular estrous cycles, known uterine compromise and/or retain fluid after breeding	4 times per day after hCG administration