

“So Many Mares, So Little (*normal*) Semen”

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In our practice area it is not uncommon for breeding farms to limit their use of veterinarians to palpation of mares to screen for breeding and utilize lay personnel for collection and processing of semen for insemination and transport. Some farms do this to reduce costs, while others seem to think that if they own a stallion they are supposed to know all there is to know about being a “stud man.” The lay expertise varies from highly trained experienced individuals to inexperienced self-taught stallion owners. Stallion fertility must be thought of as directly correlated to owner virility and is not discussed much if not better than average. Furthermore, as veterinary practices compete for the mare work, as well as the pediatric and general medicine that comes with the account, farms exert pressure to receive stallion help for access to the outside mare owners business. The bottom line is that we don’t always get a chance to market the expertise we garner from meetings such as this for a well deserved professional fee until stallion management is in enough trouble to ask for badly needed help. This presentation is simply a story of how I was fortunate enough to use the opportunity to help diagnose the cause of low conception rates in a breeding program to turn an average account into a real good account by employing some of the techniques commonly employed in reproductive practice today. Nothing we did will be groundbreaking or new to this audience. Data is not intended to be presented as excellent basic science. I simply present it as a stimulus for discussion of how you might have handled the situation and how you market your reproductive services in a competitive market.

The situation was this. Shortly before the 2002 breeding season I was asked to work for a Quarter Horse ranch standing 3 stallions. A 12 yo stallion that was a leading sire in his facet of the industry had the largest book. Due to the number of mares booked and difficulty in settling mares with cooled transported semen the two previous years, his book was limited to on- farm mares only. The two other stallions are sons of his and stand to respectable but smaller books comprised of mainly off farm mares to be bred with cool transported semen. The stallions were home bred and owned and the ranch owner collected and processed the semen. I was to palpate mares every other day to identify possible breeders and problem mares needing treatment. The owner would collect and inseminate mares according to the collection statistics. I was aware that the main stallion might have reduced fertility because a client of mine had booked mares to him the previous year that failed to conceive. The stallion owner seemed knowledgeable and conscientious and felt that closing the horses book to shipped semen and using more sperm cells to breed resident mares would improve fertility. He declined the recommendation that we perform pre season breeding soundness exams on the stallions. There were numerous dry mares already on site under lights in addition to the ranch- owned mares and the mares to be foaled out and bred back. It appeared there would be plenty of work to make a regular stop, so I agreed to take on the account.

I started making regular visits to the farm in late January to work up barren mares and become familiar with the facility and routine. The facility was well designed and the staff was organized and efficient,

always welcome discoveries when facing the prospect of a busy breeding season. We started checking mares on an every- other- day basis beginning February 2, including nine mares that had been “test bred” early. Of nine cycles six were pregnant with six being bred from one collection and I was beginning to think my concerns of stallion fertility were not warranted. Twelve to fifteen mares were presented per breeding day. The light program seemed to be effective as we were identifying cyclic mares to breed that were responding to induction of ovulation. Mares were ovulating by the next breeding day and were streaming through at a steady but not hectic rate of 3-4 per collection. The first two weeks (7 breeding days) of pregnancy checks produced 12 pregnancies from 22 cycles for a per cycle conception rate of 54.5%. That group included a few barren mares and 3 foal heat mares so that rate was not too alarming. However there were unexpected mares in the open group. The last week of February gave cause for concern by producing 3 pregnancies from 15 cycles, to finish the month with 21 pregnancies from 46 cycles for a per cycle conception rate of 45.6%. Examinations performed on mares that checked open revealed few valid reasons to explain the conception rate. Stallion management demonstrated little concern in spite of my opinion that there appeared to be no apparent problem on the mare side.

March began with the breeding season gaining momentum. Twenty to thirty mares were being presented for consideration every other day. Given the results of the last week in February, I began to choose mares to be bred with increased scrutiny. This was an effort to reduce any deficiencies on the mare side of the equation and did provide an opportunity to do mare breeding soundness exams as mares checked open, but I was hesitant to penalize mare owners while being apprehensive about stallion fertility. We started palpating mares on the off collection day and inducing ovulation in an effort to shorten the breeding to ovulation interval. The mares we chose to breed were then checked again immediately before collection to insure they hadn’t ovulated prior to breeding. Susceptible mares or any mare with evidence of a post breeding endometritis after ovulation were treated as necessary. These mare management changes made no difference in conception rates as we had 6 pregnancies for 18 cycles the first two weeks of March, and maintained roughly a 30% per cycle conception rate the rest of the month achieving 14 pregnancies from 45 cycles. Pregnancies seemed to occur somewhat at random without regard to the classification of the mare, although we had discontinued breeding foal heat mares by then. The stallion owner maintained that he was inseminating with a minimum of 1 billion motile sperm, approximately double that of a standard insemination dose. Numbers of mares bred per collection varied from 1 to 5 with a range of 2-8 billion total sperm per insemination dose. Pregnancies per collection ranged from 0/1 to 3/5. As March came to an end there were 35 mares in foal out of over 100 booked and several mares were open after 2 or more cycles. At this point my input was limited to stating that 30% per cycle was not a desirable conception rate and that from my perspective we didn’t have that many problem mares. Mare owners started to inquire about the fertility of the stallion putting stallion management on the defensive. Any who have experienced this scenario know that morale around the breeding farm sinks to a level that makes the experience unpleasant. Two consecutive 0 for 2 pregnancy check days finally penetrated the shield of denial sustained by the stallion owner much like a substance abuser “hitting bottom.” He asked me to see if I could determine the problem and offer a solution. This allowed the opportunity to market some services and expertise on the stallion side of the process that wasn’t available before.

We started with a Breeding Soundness Exam on the stallion as well as an evaluation of the entire process of collection and semen handling. Exam findings were as follows: Good breeding condition; actively breeding, considered at DSO with 35 ejaculations in last 60 days; Normal penis with a moderate

deviation to the right when tumescent; one scrotal testicle with normal orientation, consistency, and ultrasonographic appearance with a computed volume of 308.3 cc (abdominal testis reported to have been removed at 4 years of age); predicted DSO from volume of 6.1 billion spermatozoa; tail of epididymus larger than expected normal and oriented more dorsal toward the center of the testis than the posterior pole, not painful to palpation; internal genitalia was not examined at this time. The stallion was collected by the owner on a breeding phantom with a Colorado style AV after utilizing two mares in behavioral estrus confined in breeding stocks as a teasing stimulus. The stallion exhibited normal libido, mounting, intromission and ejaculation ability, although two mounts were required to achieve ejaculation.

Semen evaluation was as follows:

Volume-	67ml gel free
Concentration	207×10^6 /ml
Total Spermatozoa	13.87×10^9
Motility-extended	50%total/ 35%progressive / moderately fast velocity

Sperm Morphology – wet mount in Buffered Formol Saline with Differential Interference Microscopy by Dr. Varner

% Normal	18
% Abnormal Heads	39
% Abnormal Acrosomes	3
% Tailless Heads	2
% Proximal Droplets	8
% Distal Droplets	10
% Abnormal Midpieces	22
% Bent Midpieces	4
% Bent Tails	32
% Coiled Tails	4
% Premature Germ Cells	2

We froze a raw semen sample and submitted it for **Sperm Chromatin Structure Assay** by Dr. Love. The subject stallion had a COMP α -t of **30%** compared to a COMP α -t of **6%** for the fertile control stallion indicating that he had a much higher percentage of cells falling outside the main population than a stallion of known good fertility.

The lab was well equipped, organized, and clean. Labware and AV component cleaning and storage were acceptable with the exception that it was recommended that a water deionizer be installed for rinsing due to impurities in the tap water.

The collection area and procedure were good. I reminded the collector that it is better to change collection bottles if more than one mount is required due to the potential build up of lubricant in the collection container. I felt the motility estimate of the collector was at least 15% too generous due to non-progressive motility.

Different extenders and extender/antibiotic combinations were compared to the extender being used. It was found that the extended motility velocity and longevity for this stallion were improved in a dual sugar glucose/sucrose nonfat dry skim milk based extender with Potassium Penicillin 1000 IU/ml and Amikacin 1 mg/ml over the extender that was being used.

I sought and received an excellent consultation from Doctors Varner and Love. With the abnormal aspects of the semen morphology and abnormal DNA indicated by the Sperm Chromatin Structure Assay there was concern that increasing the sperm in the insemination dose may not have a positive affect because infertile spermatozoa could be motile and compete for sites in the oviductal epithelium. Horses with chromatin structure abnormalities as numerous as this horse's sometimes settle mares somewhat at random and increasing the insemination dose has little effect on fertility.

To determine if we could have positive effect on fertility with increased insemination dose, we chose to do a two-week trial breeding no more than two mares per collection. Prior to the trial there had been 35 pregnancies from 91 cycles for a per cycle conception rate of 38.5% for the season. The stallion owner continued to collect the stallion and inseminate the mares at this time. The 14 cycles during the trial produced 9 pregnancies for a 64.3% rate, indicating that more sperm per insemination dose may make a difference. By the time we compiled this information we were at the beginning of May with 43 mares pregnant of a 110 mare book. Although we were settling a better percentage of mares per cycle we weren't going to get them all pregnant by a reasonable end date to the season by breeding them two at a time. Therefore, since we showed a positive effect from increased numbers of sperm, we would try to breed more mares again but use a deep horn insemination technique to see if placing the sperm as near to the oviductal papilla as possible would have as positive effect as doubling the number of sperm inseminated.

On May 4, I began breeding 4-5 mares per collection by a rectal guided deep horn insemination technique. The 12 days until the first pregnancy checks for these breedings passed slowly with much anxious anticipation. When the day arrived to check the 4 mares that had been bred the first day of the trial were waiting in the stocks when I arrived at the farm. To our delight, all four were pregnant! Two days later 5 mares of 5 checked were pregnant. With each pregnancy the morale of the breeding crew improved immeasurably. I would like to say the rate kept up at that rate, but that would be too good to believe. It did hold up to achieve 27 pregnancies from 48 cycles bred in May for a 57% per cycle conception rate. Insemination doses ranged from 1.28 to 2.6 billion total sperm per dose with a volume of 10 to 25 ml. Thus we had returned to the rate at which we were breeding mares in March but with twice as many pregnancies. Also we reached the 80 mares in foal mark, which made it possible to see the 100 mares in foal mark, which we did reach by July 10.

Also, by the first of May the stallion a little fatigued and his libido decreased some. The owner started to have some difficulty in collecting him, as well as, one of the other stallions. We were charging an hourly rate to stay while they were collecting the horse any way, so the owner saw the benefit of having me collect the horses and process the semen. I was able to collect both horses with good consistency, and we were getting the mares in foal, so the owner felt justified in the added expense of having us do so. Thus I'm doing more work, he is obtaining better results, the mare owners are getting their mares in foal, and everyone is happy.

We started the 2003 season with the same protocol with the addition of daily palpations and inducing ovulation the day prior to breeding in all the mares bred. By checking mares daily and making the distinction between mares ovulating within 24 hours of insemination and those ovulating between 24 and 48 hours from insemination, we were able to determine two different per cycle conception rates for the two groups. Those mares ovulating within 24 hours of breeding conceived 62% of the time. Mares ovulating between 24 and 48 hours of ovulation had a 44% per cycle conception rate. This relationship stayed consistent throughout the season this past year. The increase in pregnancy rates justified the added expense of daily palpations. Since we gained the owners confidence at the end of the year in 2002, we collected the stallion from the beginning and throughout the 2003 season, providing regular semen evaluation of every ejaculation. By achieving a better conception rate from the beginning of the season, we were able to obtain a 96 % seasonal pregnancy rate much earlier in the year. Mare owners got their mares in foal earlier and back home for less cost restoring their confidence in the farm and salvaging the stallion's reputation in the industry. Therefore I generated more income from veterinary services billed and the client generated more income from stud fees collected; a beneficial relationship for both parties involved.