

EMBRYO TRANSFER DOWN SOUTH

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I was generously invited by a friend and colleague, Dr. Cezar Bergada, to come down to South America and see what embryo transfer services are like in his native country of Argentina. So in late January, my wife and I began a 12-day adventure, eager to explore a few facets of Argentina's horse breeding industry.

Our first stop was to Santa Margarita, a prominent Quarter Horse breeding and training farm 1 hour south of Buenas Aires. Dr. Bergada and Dr. Marianna Calderon are the two staff veterinarians at this facility. We were greeted with a grand tour, seeing the beautiful layout of the various training barns, offices, houses and new on-site surgical clinic. But as one would expect, we eventually gravitated towards the breeding barn where we spent most of our time. This barn was well equipped with the essential materials needed for breeding. There was a semen collection area with phantom, a set of stocks for palpating and breeding mares and a full laboratory for semen analysis and processing. An interesting thing to remember is that Argentina's breeding season occurs primarily during the months of November through March. Being in the southern hemisphere, their summer months are considered the winter months for the northern hemisphere. Because our visit was at the end of January, their breeding season was still very active.

Approximately 100 mares are bred at this facility each year with the resulting foals being raised and then either sold as yearlings or trained for cutting and reining. Just about all of these mares are bred artificially with fresh semen collected from any one of 6 or 7 stallions standing at the facility during the breeding season. The use of cooled transported semen is not as common in Argentina as is in the U.S. Apparently, many horse owners and veterinarians do not know how to properly use cooled semen. In addition, most of the equipment needed only comes from the U.S.

High import taxes make it impractical for many horse owners and practitioners to get started. At one point, Dr. Bergada and I were discussing the possibility of utilizing Equine Reproduction Concepts (ERC) to ship cooled semen to Argentina from a prominent stallion now showing in the U.S. It is possible to get a package from the U.S. to Argentina within 24 hours. However, shipping semen across international borders is always more complicated because of import/export guidelines for biological substances.

Dr. Bergada spends time in the U.S. every year going to different facilities and learning techniques that can be implemented into his own situation. The last couple of years, he has started a small embryo transfer program whereby anywhere from 4 to 6 Quarter Horse mares are entered as embryo donors. While at Santa Margarita, I had the opportunity to watch and participate in their procedures for managing the recipient mare herd as well as observe their methods for embryo recovery and transfer. The procedure for recovering embryos is typically the same no matter where you go or who is doing them. It's simply placing a catheter into the vagina and through the cervix so a modified saline solution can flow to the uterus. The idea is to suspend the embryo in fluid and then recover the fluid along with the embryo back through the catheter and into a filter system. A certain technique is necessary to make sure the fluid reaches all sections of the uterus and subsequently almost all of the fluid is evacuated.

Whether it is the embryo recovery process or the transfer procedure, there are certain techniques that have to be tailored to an individual's needs and likes. Dr. Bergada is still trying to finalize an embryo transfer procedure that he is comfortable with. Although ERC utilizes both a surgical (flank incision) and nonsurgical (via the cervix) technique for

transferring embryos, the surgical procedure is presently the method of choice. This however is impractical for small operations such as Santa Margarita, so the nonsurgical approach becomes more readily adopted. Historically, pregnancy rates have been 10 to 25% higher utilizing the surgical procedure but in recent years nonsurgical techniques have been mastered to the point where pregnancy rates are reportedly similar. One of the interests in coming to Argentina was to observe the techniques of two prominent commercial embryo transfer centers who routinely have reported nonsurgical pregnancy rates at or above those rates for surgical procedures.

Our first trip to an embryo transfer center took us northwest of Buenos Aires to Dr. Fernando Riera's facility. It was very interesting because before the clinic was even in sight we ran across several acres of corn fields with horses grazing within the corn stalks. It took me by surprise because I had never seen horses turned out to graze on corn stalks. I soon found out that all donor and recipient mares live together out in the fields. Their turnout is rotated throughout the year between various types of crops; hay pasture and sorghum grass in the winter and early spring, corn in the summer months and soybean in the summer and fall.

We approached the clinic and found ourselves walking in right at the perfect time. Dr. Riera was in the process of preparing for 4 embryo recovery attempts. Almost all of the donor mares in his ET program were mares that had just finished the polo season. Prior to our arrival, 50 recipients were already pregnant with another 20 awaiting an exam to determine if they were pregnant or not. Over 120 recipients were still open and available for transfer.

Interestingly, Dr. Riera had modified his embryo recovery procedure so that a single person could perform the procedure without any assistance. He uses a continuous catheter system which simply utilizes a Y-junction at the end of the uterine catheter to divert the modified saline solution into the uterus and then subsequently out through another tubing system connected to the capped, embryo filter cup. A prefabricated metal

bracket hangs from the back of the stocks to support the filter cup as fluid is retrieved. I was impressed with the efficiency the procedure created both in time and labor. At ERC, we routinely use an open, noncontinuous system whereby the fluid flows into the mare and then we detach the tubing and reconnect to another piece of tubing for proper filtration. This procedure does require another person to oversee the fluid flow through the filter cup. Aside from the practical aspects of the modified catheter system, Dr. Riera's technique was similar to ours in that rectal manipulation of the uterus was used periodically for fluid redistribution while 2 to 3 liters of saline media was intermittently passed into and out of the uterus. The 4 recovery attempts we were present for yielded 6 embryos. Obviously, double ovulations helped with his recovery rates.

After each embryo was recovered and evaluated for quality and size, Dr. Riera performed a rather interesting type of nonsurgical transfer procedure. The technique he has the most confidence in consists of both vaginal and rectal manipulation. First he passes the sterile, sheathed implantation pipette into the vagina and through the cervix. Then by rectal manipulation he guides the tip of the pipette up a uterine horn and deposits the embryo. Most practitioners tend to use a more simple approach by remaining in the vaginal cavity and passing the pipette straight through the cervix to the uterine body where the embryo is deposited. Dr. Riera's embryo recovery and transfer techniques have proven to be very successful. After examining his records, I found that 91 of 105 (86.7%) recovery attempts yielded an embryo. These results are exceptional. Based on data from various studies and embryo transfer centers, recovery rates were typically found to be between 60 and 70% successful when normal mares are bred with viable semen. It must be pointed out that several factors affect embryo recovery rates. Characteristics such as age and reproductive soundness of the donor as well as quality of semen inseminated all have a dramatic effect on the success of an ET program. Because most of Dr. Riera's donor mares are active in polo they tend to be younger in age. In addition, these mares are bred with fresh semen from a few

stallions that have already proven themselves to be quite fertile. These characteristics do help to optimize results for his ET program but let's not disregard the importance of experienced personnel performing the procedures. During the 1999 season, we at ERC recovered embryos from 70% (35 embryos/50 attempts) of the attempts. We were very pleased with these results since the donor mares in our program ranged in age from 4 to 23. In addition, odds were against us a little because fresh, cooled and frozen semen were all actively being used to breed these mares.

Dr. Riera's embryo transfer rates were equally as impressive as his success with recovering embryos. Of the 73 embryo transfers that were performed prior to our arrival, 54 resulted in a pregnancy (73.9%). Nonsurgical transfers have historically been anywhere from 40 to 60% successful bowing to the superior pregnancy rates of surgical transfers (60 to 75%). In asking what Dr. Riera believes is the key to his success, he was quick to point out a few factors. Along with having found and perfected certain techniques that he believes in, the fertility of the donor mare and stallion play a big role. In addition, the condition of the recipient mare is also of vital importance. He strives to use young, preferable maiden mares that have been maintained on an adequate nutritional diet. This sentiment was echoed by Dr. Victorio Toto who was resident veterinarian at the second embryo transfer program we visited. Also in northern Argentina, this operation was attractively nestled within 2000 acres of pasture and cropland. A total 450 horses were at this facility which consisted of 30 donor mares, 200 recipients and the remainder being other brood mares and horses in training for

polo. The recovery and transfer procedures performed by Dr. Toto were almost identical to those done by Dr. Riera. According to Dr. Toto, success rates were also very similar; yielding an average seasonal recovery rate of 85% with approximately 75% of the transfers resulting in a pregnant recipient. In addition to the keys of success mentioned earlier, Dr. Toto believes that some of the credit belongs to the fact that the donor and recipient mares are housed together, faced with the same environmental conditions.

Although the success of these embryo transfer programs was very impressive, it became clear to me that Argentina's commercial embryo transfer industry is based on different interests than those seen in the United States. These interests seem to have an indirect influence on the success of the program. To clarify, the typical client involved in an ET program in Argentina is interested in getting multiple pregnancies from mares, presumed younger in age, that are still active in competition. Because the availability or interest in cooled and frozen semen is relatively non-existent, breeding is almost exclusively done with fresh semen. Most people involved in a U.S. embryo transfer program are striving to obtain only 1 or 2 pregnancies from mares that may be young or old, with or without reproductive problems. In order to get the best genetic cross possible, many clients use cooled and frozen semen even though embryo recovery rates may be affected. Another interesting difference is that many breeds of varying disciplines are involved with ET programs in the U.S. Argentinean ET centers are primarily structured for the polo mare with minor involvement from show jumpers and other disciplines.