How Embryo Age and Recipient Asynchrony Affect Pregnancy Rates.

-Samantha Brink

The equine embryo transfer program has great emphasis placed on the degree of synchrony of ovulation between the donor mare and recipient mare. Throughout the industry it has been accepted that the pregnancy rates are optimal for recipient mares having ovulated 1 day before (+1) to 3 days after (-3) the donor mare. Because of this window of synchrony, most commercial embryo transfer programs monitor multiple recipients for each donor to make sure there is a recipient that will ovulate within this window. The age or stage of the embryo development also has an effect on when it can be transferred and have good pregnancy rate. If you had transferred an embryo from in a mare that just ovulated into a mare that is close to ovulation the pregnancy rate would not have been good but now we have studies that prove you don’t have to be within those +1 to -3 days. Below are some studies and data that prove this.

Wilsher and Allen transferred day 10 (ovulation) embryos to recipients that had ovulated 3 days before transfer and observed that the embryos had heartbeats in 37.5% of the mares [Wilsher and Allen]. In a similar study, pregnancy rates were achieved between 100% and 63% when transferring day 10 embryos to recipients with degrees of synchrony varying between +2 and -6 days. The time of the uterine flush in relation to the day of ovulation is one of the most important factors in determining the success of recovering an embryo from the donor mare. The embryo remains in the oviduct and usually doesn’t enter the uterus until day 6, therefore the flushes that are preformed before 6 days post ovulation more than likely result in no recovery of the embryo. The flushes that are done on day 6 will have a significantly lower rate of embryo recovery than if the flush is preformed after day 6. It is current practice to preform the embryo recovery flush on days 7 or 8 post ovulation for embryos that will be cooled for short-term storage or immediately transferred. The older embryos (day 9) will have increased fluid volume-to-surface ratio and be more susceptible to damage during the procedure.

Pregnancy rates after transfers of embryos are also affected by the day post ovulation of the recipient mare. Pregnancy rates tend to differ when transferring day 7 or 8 embryos into recipients between days 5 and 9 post ovulation. Age and size of embryo also can affect pregnancy rates; the rates are significantly lower for smaller day 7 to 8 embryos with diameters of 100-299um when compared to more developed embryos of the same age range but diameter greater than 300um. It was reported that there were no pregnancies after transfer of day 9 and 10 embryos, where pregnancy rates of 61%, 55% and 25% were observed after transfer of days 6, 7 and 8 embryos. [Vogelsang et al]

The data from this study was obtained during four breading seasons from 2006-2009 in Seropedica and Rio de Janerio, Brazil. There were 118 donor mares, which were the Mangalarga Marchador breed, they weighed between 836-1,045lbs and were between 3 and 23 years old. The mares were fed timothy/alfalfa mixed hay and were given a commercial concentrate ration at a rate of 1% body weight per day. Mineral salt and fresh water were provided ad libitum. Using transrectal palpation and ultrasonography they followed the ovarian activity of donor mares. During estrus, the ovaries were scanned daily to determine the day for induction of ovulation as well as the day of ovulation. The criterion that was used to determine the time of induction were: presence of follicle with diameter greater than or equal to 35mm, a relaxed uterine & cervical tone, and endometrial edema consistent with estrus.

Thirty-one Mangalarga Marchador were also used as the breeding stallions, ages 4-23 years and had proven fertility. The semen was evaluated at the beginning of each breading season for progressive motility, vigor, concentration and pathology. The inseminations were preformed with fresh or cooled semen with a minimum amount of 500 million viable sperm approximately 24 hours after induction of ovulation. If no ovulation occurred then hCG was administered and inseminate again 48 hours after the first AI.

There were 560 recipient mares between 3-12 years old and weighed between 770-990lbs. These mares were fed pasture grass supplemented with alfalfa hay and concentrate; mineral salt and fresh water were available ad libitum. Estrus cycles were also monitored using transrectal palpation and ultrasonography to synchronize ovulation between the donor and recipient. The ideal degree of synchrony between the donor and recipient mare range from +1 to -6. Mares that got selected as candidates for receiving the embryos took these into consideration: degree of synchrony with donor, acceptable uterine and luteal echosities, and uterine and cervical tone compatible with diestrus.

Uterine flushes were used for recovering 809 embryos, the flushes were preformed between days 6 and 10 post ovulation and the day of recovery was chosen at random for mares.
with single and double ovulations. Availability of the operator(s) and recipient mares within a reasonable range of synchrony was also put in consideration for choosing the mares. The mares were restrained and the perineum cleaned with mild soap and iodine solution, then a 32 French silicon Foley catheter (Bioniche Animal Health, Canada) was inserted into their vagina via cervix. The catheter was inserted manually, once the catheter was in the uterine body a cuff on the end of the catheter was inflated with 40mL of air then pulled caudally to make sure the seal was tight. Lactated Ringer’s solution was infused through the catheter then into uterus and immediately drained into a collection cup with 75um filter. This was preformed 3 times and during the last flush the uterus was massaged transrectally to help cause the suspension of the embryo in flushing medium and remove as much fluid as possible. After the uterus was drained the catheter was removed there was about 50mL of solution left in the collection cup, it was placed in a petri dish and analyzed under a stereomicroscope. The retrieved embryos were subjected to 10 washes to remove cellular debris; they were then graded morphologically according to quality 1 (good) -4 (bad).

The recipient mares were chosen to receive embryos if they were within +1 to -3 window of synchrony, if there were no mares in that window the next were -4 to -6 degree of synchrony. Before transfer, each washed embryo was put into a sterile AI pipette with at least 0.5mL of holding medium. The pipette was entered transvaginally into the cervix, and precautions were taken to make sure there was minimum to no contamination during the operation. Not all the recovered embryos were transferred due to loss during handling, poor quality, freezing or lack of ready donor on the day of recovery. Pregnancy in recipient mares was evaluated using transrectal ultrasonography and examinations were done 15, 30, 45 and 60 days post ovulation. Data was not available after 60 days because most mares left the facility by then. Embryo loss between days 15 day 60 could not be evaluated due to unavailability of data from earlier pregnancy checks.

### Table 1

<table>
<thead>
<tr>
<th>Day of flush (Day 0 = ovulation)</th>
<th>Recovery rate (%)</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>42*</td>
<td>16/38</td>
</tr>
<tr>
<td>7</td>
<td>61*</td>
<td>159/262</td>
</tr>
<tr>
<td>8</td>
<td>66*</td>
<td>285/434</td>
</tr>
<tr>
<td>9</td>
<td>59*</td>
<td>39/65</td>
</tr>
<tr>
<td>10</td>
<td>56*</td>
<td>5/9</td>
</tr>
</tbody>
</table>

a,b Within a column, values without a common superscript differ (P < 0.05).

During the 4 breeding seasons, 809 flushes were preformed with 504 embryos. Days 7, 8, 9, and 10 had similar rates of embryo recovery, but day 6 was lower compared to the others. (Table 1). According to synchrony the pregnancy rates had no significant differences among days +1 to -5 but on day -6 there was a lower pregnancy rate (Table 2). A total of 545 embryos were transferred to recipients between 2 and 8 days post ovulation. Overall pregnancy rate at 60 days was 60%, the recipients that had transferred on day 2 had a lower rate when compared with transfers on days 3 to 8; and day 7 had slightly higher rates than days 6 and 8 (Table 3). The effect of pregnancy rates due to age of the embryo was that day 6 embryos produced fewer pregnancies than day 7, but no differences were seen between days 6, 8 and 9. Day 7 had a higher rate, day 9 had a lower rate and day 10 had no pregnancies. [Jacob et. al. 2013]

In traditional embryo transfer in the equine industry it is said that the optimal time to transfer an embryo would be when the recipient has ovulated 1 day before to 3 days after the donor mare ovulates. Based on this study, the transfer window does not need to be quite as restricted. An acceptable pregnancy rate, about 70%, was obtained when the recipient ovulated 4 to 5 days after the donor mare. Most programs monitor multiple recipient mares for one donor mare so the degree of synchrony between the mares will be within the generally accepted +1 to -3 day window. Now because the window could be extended to as far as the recipient can ovulate 5 days after the donor mare so fewer recipient mares need to be monitored to achieve the same pregnancy rates. Three day 9 embryos were transferred to recipients 2 days post ovulation and resulted in 100% pregnancy rate, reinforcing the notion that pregnancies can be produced even in extreme degrees of asynchrony when recipient’s uterus has experienced minimal progesterone exposure. One of the most time consuming parts of preforming embryo transfer would be examining donor and recipient mares to determine their ovulation dates and degrees of synchrony. If...
they didn’t have to monitor as many recipients that would greatly benefit any equine embryo transfer program. They would have to feed fewer mares, less reproductive examinations, lower costs for hormonal treatments and a reduced need for manual labor.

With the current practice to recover an embryo via uterine flush on days 7 or 8, flushing before day 7 may result in lower recovery rates even though comparison of uterine and oviductal recovery shows the embryo is in the uterus by day 6. The current findings from this study agree that day 6 recovery rate of 42% was significantly lower than the later days. Similar recovery rates of 63% were observed when preforming the flush between days 7 and 10, even though the increased fluid volume-to-surface ratio makes an older embryo more susceptible to damage during the flush. Days 9 and 10 can be retrieved with success rates comparable to the flushes on days 7 and 8, which shows that day 10 embryos can produce acceptable pregnancy rates.
Work Cited:

