New technics of artificial insemination in the mare (15-Nov-2003)

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Timing of Insemination

The lifespan of spermatozoa in the female reproductive tract determines the maximum interval from insemination to ovulation that will provide the maximum fertility. Once the spermatozoa enter the uterus, they migrate to the oviduct where they bind to the oviductal wall. The oviduct acts as a reservoir and spermatozoa are released gradually from the oviductal epithelium into the oviductal lumen and this over an extended period of time. There is evidence that frozen-thawed spermatozoa do not bind as well to the oviductal wall and are lost from the oviductal reservoir sooner than fresh spermatozoa. Based on this information it would seem logical that mares bred with frozen semen should be inseminated closer to ovulation. Because of the belief that frozen semen has a shorter lifespan in the mare, most breeding protocols for frozen semen attempt to inseminate as close as possible to ovulation. Fresh semen remains viable in the oviduct for several days. Based on many studies, insemination with frozen semen should be performed within 24 hours before ovulation or within 6 hours after ovulation. Barbacinni (European Select Breeder Services – Italy) reported on 1,040 cycles inseminated with frozen semen within 6 hours before (363 cycles) or after (677 cycles) ovulation. Per-cycle pregnancy rates were similar for pre (41%) and post (41.3%) ovulation.

For many stallions, the number of insemination doses per mare is limited and veterinarians are asked to get mares pregnant with as few doses as possible. This forces the veterinarian to examine the mare every 6 hours and to inseminate the mare with a single dose after the mare has ovulated. Although the pregnancy rate is the same as that obtained with insemination before ovulation, the cost to the owner and the workload for the veterinarian are significantly higher.

Observations made in the Haras Nationaux in France indicate that two or more inseminations per cycle result in higher pregnancy rates as opposed to mares that were inseminated only once. This has been observed for fresh semen (daily insemination until ovulation is detected) but also for frozen semen. This observation suggests that when adequate number of doses are available, mares should be inseminated twice, if possible, with the last insemination occurring within 24 hours before ovulation. In this context, Barbacinni reported on the insemination with frozen semen 24 hours and 40 hours after hCG administration (ovulation occurs on average 36 hours after hCG). Pregnancy rates with the timed insemination scheme were similar (76%) as those obtained with a single insemination after ovulation (71%). Similar observations were made by the Colorado group. The advantage of the fixed time insemination scheme is the reduced workload, once-a-day examination versus 4 times-a-day examinations at 6-hour intervals.

However, these conclusions give rise to several new questions that researchers have recently tried to address. Does the inflammation and influx of inflammatory cells in the uterus compromise the potential fertility of the second insemination when performed within 24 hours after the first? If only one dose of frozen semen is available per cycle, can we still use the timed-insemination protocol?

Single versus multiple inseminations per cycle.

Following insemination, spermatozoa migrate into the oviduct where they will be stored until ovulation. It is believed that the oviductal storage represents also a selection period during which only good quality spermatozoa are retained in the oviductal crypts and lesser quality spermatozoa are released prematurely (before ovulation) and thus not available for fertilization. Studies have shown that all viable spermatozoa (those apt to fertilize the oocyte) are within the oviduct within 4 hours after insemination. Uterine lavage with a spermicidal, diluted povidone-iodine solution 30 min, 1 or 2 hours after insemination.
decreases pregnancy rate in normal fertile mares whereas uterine lavage 4 hours after insemination results in pregnancy rates that are the same as those obtained in mares that were not lavaged. This leads us to conclude that all residual material (spermatozoa, seminal fluid) still present in the uterus 4 hours after insemination does not contribute to the success of the insemination.

Insemination using fresh semen initiates an acute inflammatory reaction in the uterus. Numerous white blood cells enter the uterus in an effort to clean the uterus from the foreign material that has been introduced. This inflammatory reaction is exacerbated after insemination with frozen-thawed semen. Studies have shown that the seminal plasma (which is removed during cryopreservation) has an inhibition on the post breeding uterine inflammatory reaction. Seminal plasma has an immuno-suppressive effect in the uterus. Elisabeth Metcalf (AAEP 2000) examined the effect of this post-breeding endometritis on the fertility of a subsequent insemination. In her study, mares were inseminated twice at 6 - 10 hours interval. At 34 - 40 hours after hCG administration, mares were inseminated for the first time with frozen semen from one stallion. After ovulation, mares were inseminated again with frozen semen from a different stallion (interval between first and second insemination ranged between 6 - 10 hours). Paternity testing of the foals demonstrated that as many foals originated from the first insemination as from the second insemination indicating that the post breeding endometritis initiated by the first insemination did not affect the fertilizing capacity of spermatozoa introduced during the second insemination. Thus, we can conclude that a second insemination can safely be performed within 24 hours after the first insemination and that insemination within 6 hours after ovulation results in acceptable pregnancy rates.

Researchers in the Haras Nationaux (France) reported that when mares were inseminated on 3 subsequent days with fresh semen from 3 stallions (one stallion each day), the first insemination has the same likelihood of fertilizing the oocyte as the third insemination. From this data, we can conclude that previous inseminations do not have a detrimental effect on the present insemination and that (fresh) semen can persist for several days (3 in this case) in the oviduct and maintain a normal fertilizing potential.

Some concerns may exist when dealing with mares with excessive endometritis and/or fluid pooling. A recent study by Vanderwall et al., reported that uterine lavage with Lactated Ringer Solution (4 x 1 liter) immediately before insemination does not decrease pregnancy rates. The authors concluded that the inflammation induced by the lavage did not adversely affect the viability of the spermatozoa or their fertilizing capacity.

The above studies and the numerous reports on timed-insemination protocols demonstrate that multiple inseminations in normal mares are not harmful. Moreover, data collected over several years in the Haras Nationaux (France) suggests that multiple inseminations (fresh or frozen) result in higher pregnancy rates. These observations argue in favor of timed-insemination protocols such as insemination 24 and 40 hours after hCG administration. However, when the number of doses per mare are limited, this method is not well accepted by mare and stallion owners.

**Insemination Dose**

In recent years, insemination with less than the standard dose has received increasing attention. In the Haras Nationaux (France), a standard insemination dose of fresh semen consists of 200x10⁶ total spermatozoa. Using frozen semen, a standard dose represents 400x10⁶ total spermatozoa with a post-thaw motility larger than 35%. Using the French system, frozen semen is conditioned in 0.5 ml straws at a concentration of 100x10⁶ spermatozoa/ml (50x10⁶ spermatozoa/straw). Thus, one insemination dose represents 8 straws containing 50x10⁶ spermatozoa each. In the USA, insemination is done with 500x10⁶ spermatozoa that are progressively motile (PMS) using fresh semen and 800 to 1000x10⁶ spermatozoa when using frozen semen.

With the development of international trade in frozen semen and the decreasing availability of insemination doses from top stallions, more people have looked for methods to reduce individual insemination doses. In parallel, the development of sex-sorting semen and the practical application of sex-sorted semen in equine reproduction has necessitated the development of techniques that allow the successful insemination of very small amounts of semen. Theoretically, the development of techniques for low dose insemination would allow one to increase the number of mares that could be bred, utilize stallions with poor semen quality, extend the use of frozen semen, used sex-sorted semen, even reduce the incidence of post-breeding endometritis. Three low dose insemination techniques have been described: surgical oviductal insemination, deep intra-uterine insemination and hysteroscopic insemination.

Using oviductal insemination, via surgical exposure of the ovary (laparotomy) or via endoscopic approach (laparoscopy),
Researchers were able to obtain acceptable pregnancy rate with as little as 200,000 fresh spermatozoa placed into the oviduct. The reported pregnancy rates were the same as those obtained in control mares inseminated with 500x10^6 spermatozoa. However this method is invasive and costly and is therefore not acceptable for application on client animals. Moreover, studies on GIFT suggest that deposit of cooled or frozen-thawed semen directly in the oviduct does not result in pregnancies, possibly due to components in the semen extenders used for cooled-storage and cryopreservation.

Several studies have examined the possibility of using videocendoscopy to deposit spermatozoa directly around or on the uterotubal papilla (the entrance from the uterus into the oviduct). It must be stressed that these experiments were done in the context of the extremely small numbers of spermatozoa obtained after sex-sorting semen. The most decisive results were obtained by the Newmarket group (Morris et al.) who was able to demonstrate that very small numbers of spermatozoa can result in acceptable pregnancy rates. They extended spermatozoa with a skim-milk extender and layered the extended semen onto a 90:45 Percoll gradient followed by centrifugation. Insemination doses of 10, 5, 1, 0.5, 0.1 and 0.001 x 10^6 spermatozoa (10 million to 1000 spermatozoa) were suspended in 30 - 50 microliters (0.03 - 0.05 milliliter) and deposited onto to the uterotubal papilla. Pregnancy rates for those mares inseminated with 1 million or more spermatozoa achieved acceptable pregnancy rates >60% whereas mares inseminated with less than 1 million spermatozoa had very low pregnancy rates. The pregnancy rates in this study were remarkable higher than in other similar studies (around 30% for similar number of spermatozoa). The authors attributed their success not only to the use of Percoll gradient centrifugation to remove seminal plasma, but also to the small volume (0.03 - 0.05 ml) deposited on the uterotubal papilla. This latter point is important in the commercial application of this technique because we are usually working with 50 x10^6 spermatozoa in 0.5 ml. This volume is at least 10x larger than the volume recommended by Morris et al. and the number of spermatozoa is 50x larger that the minimum effective number in the studies reported by Morris et al. Thus in practice this method reveals to be impractical because the volume of semen contained in one straw is too large to deposit a single drop onto the uterotubal papilla and is inefficient because the required number of spermatozoa contained in a "dose" or straw exceeds the number required.

Another method described is the insemination near the tip of the uterine horn ipsilateral to the ovary containing the pre-ovulatory follicle. This technique remains controversial. Insemination is done by advancing a long uterine insemination catheter through the cervix and then guiding the catheter by manipulation per rectum in the uterine horn ipsilateral to the pre-ovulatory follicle. The catheter used for this procedure needs to be longer than the classic AI catheters for horses, especially when working with the larger warmblood breeds (for example the deep insemination catheter manufactured by Minitub. The catheter also has to be flexible although opinions differ on this with some preferring a semi-rigid catheter to better identify and manipulate the catheter per rectum whereas others prefer a very soft catheter for fear of damaging the uterine endometrium while advancing the catheter into the horn, for example the catheter developed by the group in Gent. Whatever the material used for deep intrauterine insemination, the procedure seems straightforward and can be done with limited training for a person experienced in rectal palpation of mares.

Several researchers have examined the potential traumatic effect and negative effect on fertility of this extra intrauterine manipulation. Insemination with the same insemination dose at the cervix or at the tip of the uterine horn results in the same pregnancy rates. This indicates that the procedure in itself does not cause significant trauma and does not interfere with fertility. Several studies have attempted to demonstrate the added benefit of deep intrauterine insemination. Presently, there are very few controlled studies on the efficiency of deep insemination with reduced doses. Most of these studies suggest that acceptable pregnancy rates can be obtained with significantly lower doses of (mostly) frozen semen. Pregnancy rates with deep insemination of as little as one straw are often similar to those obtained with a full 8-straw dose in the uterine body. However, most of these studies are incomplete in that they do not provide a direct comparison between single straw insemination in the uterine body and in the tip of the horn. One study recently completed at the INRA Institute in France (Guy Duchamp 2003) compared the pregnancy rates obtained after insemination of 50 x 10^6 spermatozoa of fresh semen in the tip of the uterine horn versus the uterine body. This study indicated no difference between the two locations. At the surface, this study seems to fail to demonstrate an advantage associated with insemination in the tip of the horn. However, one needs to consider that it remains possible that when using frozen-thawed, which may be less motile and have a shorter lifespan, in difference may be observed. Further studies will need to be conducted to examine this possibility.

At the present time, review of the available data suggests that insemination in the uterine horn with limited doses of frozen-thawed semen does generate acceptable pregnancy rates when using good quality semen. However; reduction of the insemination dose in stallions of marginal fertility appears to reduce the pregnancy even further for these stallions. In conclusion, it appears that this technique may have some benefits but at the present time there are no clear guidelines or data to support the routine utilization of this technique. The only data that supports the potential beneficial effect of this insemination technique is the fact that deep intra-cornual insemination results in a significantly higher proportion of spermatozoa present on the side of insemination (presumably the side of ovulation) than in the contralateral oviduct.
Until more data is available that make it possible to generate guidelines the use of these insemination techniques needs to be accompanied by a close monitoring of the results and a constant awareness that doses need to be adapted to the fertility of each individual stallion.

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