Use of Frozen Semen in an A.I. Center: Technical Management and Obtainable Results

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The results obtained when using frozen semen are influenced by several factors including:

1) the veterinarian utilizing the frozen semen must possess extensive knowledge and experience in the field of equine reproduction,

2) much more intense mare management is required than when using fresh semen

3) the quality of the frozen semen must at least meet minimum standards. For instance, in our laboratory we prefer to use only semen that, after thawing, shows at least 30% progressive motility and contains not less than 200 - 250 million progressively motile sperm per dose.

Mare management from the moment of entry into the A.I. center:

We have omitted details of preparation of broodmares entering the breeding season and examine for the purpose of this paper only the protocol used in the reproductive management of broodmares inseminated with frozen semen. The main goal is to synchronize the moment of insemination with ovulation. Using ultrasonography with a 5 Mhz transrectal linear probe, ovarian activity is monitored during estrus using the following scheme:

- monitor follicular dynamics every 24 hours, up to the presence of one or more follicles of 35-40 mm;
- induction of ovulation by intravenous administration of 2,000 IU of HCG as soon as follicle has reached 40 mm;
- monitor follicular dynamics and ovulation induction 12 hours after the HCG administration and then every 4-8 hours up to the time of ovulation;
- insemination

Using this scheme, in the case of post-ovulation insemination, the interval between ovulation to insemination never exceeds 6 hours. Therefore, with this protocol, each insemination is made during an interval of 8 hours pre- and 6 hours post-ovulation. The frequency of monitoring follicular dynamics to establish the moment of ovulation can be modified according to the number of insemination doses available for each broodmare. If more doses are available, more doses could be employed in each estrus and less frequent could be the ultrasonic evaluation of the ovaries. We do not usually inseminate mares at the foal heat as the pregnancy rate obtainable in this

physiological phase are lower than those of successive cycles. As often times the number of doses available for each broodmare is limited (3-5), we try not to add together the decrease in fertility per cycle which could be derived from the use of frozen semen and from insemination on the foal heat. For this reason, all the lactating mares, during the foal heat are examined once per day until ovulation is confirmed and then treated with prostaglandins 5 days after ovulation in order to bring them into estrus as soon as possible. Pregnancy is verified by ultrasonography examination 15 days after the ovulation and successively repeated at 30 and 50 days of gestation.

Thawing and Insemination:
The thawing of is a very simple technique however the cells are very delicate and attention to detail is critical. Remember that by thawing we are reviving cells that until now were preserved at -196°C. As the spermatozoa must keep intact the metabolic systems required for fertilization, any mistake during thawing can intensify the stress they receive, compromising the final result. Equine frozen semen is usually packaged in straws containing 0.25, 0.5, or 5.0 ml of extended semen. The thawing is carried out using a water bath according to the following times and temperatures:

- makrotubes (5.0 ml): 50°C for 45 seconds
- 0.25 or 0.5 ml straws: 37°C for 30 seconds

After thawing, both large and small volume straws are opened at one end and the contents placed into a pre-warmed (37°C) vial. The thawed semen is then drawn into a catheter and mares inseminated as with fresh semen. It is necessary to receive from the semen producing laboratory the instructions concerning the number of straws to be utilized for each insemination as it can vary according to the percentage of progressive motility after thawing and to the sperm concentration.

Results obtained in the period 1994-1997 in our Artificial Insemination Center

During the breeding seasons 1994-1997, 293 Warmblood mares in age between 3 and 23 years were inseminated with semen belonging to 55 stallions and produced in 14 different laboratories. The total number of cycles managed by us was 576: 225 were pre-ovulation inseminations, and 351 were post-ovulation. At the first ultrasound exam (15 days) 221 of the 293 inseminated mares were pregnant, so the per cycle and seasonal pregnancy rates were 38.4% and 75.7% respectively. The number of insemination doses utilized was 593 during the time taken into consideration, with a final consumption of 1.03 doses per cycle. The number of insemination doses and cycles necessary to obtain a pregnancy was in both cases 2.6 (table 1). Data reported on table 2 shows that, by dividing the mares into groups with respect to their reproductive conditions, the total and per cycle pregnancy rates obtained on barren mares are lower than those of the maiden and lactating mares. The total and per cycle pregnancy rates are much higher in mares age 3-16 years rather than in older ones (>16 years). There was no difference in the per cycle pregnancy rates for mares inseminated pre-ovulation (225 cycles, 39.1% pregnant) or post-ovulation (351 cycles, 37.8% pregnant). Early embryonic death (prior to day 50) occurred in 21 mares (9.5% of pregnancies), divided among the reproductive groups as follows: 3 among the maidens (5%), 7 among the barren (14%), and 11 among those in lactation (10%). The incidence of endometritis, expressed as the

presence of uterine fluid of II or III degree, 24 hours after insemination, was directly proportional to age and higher in barren mares (38%) than in maiden (32%) or lactating (32%) mares. Table 3 shows the incidence of ovulations with respect to the HCG administration: it is interesting to note that nearly 94% of ovulations happened within 48 hours from the treatment and also 87.9% in the interval of 24-48 hours. Individual variation in fertility among stallions has long been recognized as a major factor affecting results with frozen semen. Table 4 illustrates this point for 13 of the stallions in our data. These are stallions whose frozen semen was used to inseminate a minimum of 13 cycles during the 4 breeding seasons. Seasonal pregnancy rates ranged from a low of 40% to a high of 93%.

Final Considerations:

It is possible to obtain high total pregnancy rates when using equine frozen semen even if the per cycle pregnancy rates are lower than those obtained with fresh semen or by natural cover. Presumably this is due to the high energetic consumption and consequent decreased metabolic potential caused by freezing and thawing equine spermatozoa. In conclusion it has been demonstrated that good results can be obtained using equine frozen semen in a commercial setting and that two very important aspects must be considered:

1) Stallions and ejaculates utilized to produce frozen semen must be properly tested to guarantee the final user at least minimum quality standards.

2) Mares must undergo intense reproductive management. If this does not occur, the results could be very low, with ruinous effects on the credibility and wide spread commercial use of frozen semen. It is mainly for this reason we recommend that, where it is possible, mares should be managed at an artificial insemination center where sufficient monitoring of follicular dynamics can occur, resulting in better results and more efficient consumption of frozen semen.