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Effect of Timing of Frozen Semen Insemination on Pregnancy Rate in Mares

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Katila, T., Celebi, M. and Koskinen, E: Effect of timing of frozen semen insemination on pregnancy rate in mares. Acta vet. scand. 1996, 37, 361-365. – Thirty-four mares were inseminated with frozen semen from one stallion during 2 oestrous cycles, every 48 h until ovulation took place and within 12 h after ovulation. Semen was frozen using the Colorado method. The insemination dose was from 200 to 400×10^6 progressively motile spermatozoa. Ovaries were examined every 12 h to determine time of ovulation. Examination for pregnancy was carried out using ultrasonography, 15 days after ovulation. Thirty-five per cent of mares inseminated < 24 h and 23% of mares inseminated between 24 - 48 h before ovulation were pregnant ($p = 0.388$). The pregnancy rate in all mares inseminated before ovulation was 30%. In the mares inseminated within 12 h of ovulation, it was 18% ($p = 0.253$). Younger mares (aged 4-10 yr) had a higher pregnancy rate (59%) than older mares (aged 11-15 yr) (23%), but the difference was not statistically significant ($p = 0.057$).

pre-ovulatory; postovulatory.

Introduction

A widely accepted explanation of poor pregnancy rates after artificial insemination (AI) with frozen stallion semen is the brief viability of frozen-thawed spermatozoa. It is therefore considered to be important to inseminate as close to ovulation as possible (*Brinsko & Varner* 1992). This requires frequent examinations of the mares.

Reports on timing of insemination with frozen semen have been published (*Palmer* 1984, *Volkmann & van Zyl* 1987, *Kloppe et al.* 1988, *Palmer & Magistrini* 1992). It is, however, difficult to conclude from these reports what the best insemination time in relation to ovulation might be. There are differences between the studies in relation to freezing techniques, insemination doses, and variations in semen fertility and freezability between stallions. It is well known that there is a tremendous variation between stallions as regards fertility of frozen semen (*Loomis* 1986, *Müller* 1987, *Brinsko & Varner* 1992).

Daily AI seems to be most common (*Loomis et al.* 1983, *Cochran et al.* 1983, *Cristanelli et al.* 1984, *Palmer* 1984, *Volkmann & van Zyl* 1987, *Kloppe et al.* 1988, *Love et al.* 1989, *Palmer & Magistrini* 1992). Insemination every 24 h before ovulation can result in more than one or 2 inseminations, since prediction of time of ovulation is difficult (*Lindeberg & Kuntsi-Vaattovaara* 1992). The procedure can accordingly be expensive, if payment per straw is involved. Inflammatory reactions after AI with frozen semen are much greater than after AI with fresh semen or natural mating, because of the high spermatozoal concentration (*Kotilainen et al.*

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1994). Repeated insemination can cause problems in mares with inadequate uterine defence mechanisms.

Satisfactory results after postovulatory AI have been obtained under practice conditions on 2 stud farms: a pregnancy rate/cycle of 43% in Sweden (*Darenius & Darenius* 1992) and 45% in Finland (*Lindeberg & Kuntsi-Vaattovaara* 1992). In Finland, a seasonal pregnancy rate of 82% and a foaling rate of 76% have been achieved but much work was needed to achieve these impressive results: 2.4 cycles/pregnancy and 23 palpations/mare (*Lindeberg & Kuntsi-Vaattovaara* 1992).

AI every other day would be economically most beneficial, because the mares to be inseminated with frozen semen could be fitted into the normal breeding routines of the stud farm. With mares inseminated from 24 to 48 h before ovulation, pregnancy rates of 5, 27, 19 and 30% have been obtained (*Pace & Sullivan* 1975, *Aliev* 1981, *Palmer* 1984, *Palmer & Magistrini* 1992). These figures are not acceptable commercially. However, *Volkmann & van Zyl* (1987) reported pregnancy rates of 55% when mares were inseminated < 24 h or 24-48 h before ovulation.

The aim of the study reported here was to examine and inseminate mares using methods that would be relatively easy and inexpensive to carry out in practice.

Materials and methods

Semen from a 15-year old Standardbred stallion was frozen between October and February. Frozen semen from this stallion had previously given satisfactory results. Thirty mares had been inseminated commercially in previous years, with a seasonal pregnancy rate of 60%. Sperm-rich fractions (one to 3) of the ejaculate were collected using an open-ended vagina (Krakow model, Poland). Only semen with > 60% progressive motility, > 150 x 10^6 sperm/ml and >70% morphologically normal spermatozoa was processed further. Concentration and motility were determined after every collection, morphology was studied monthly. Semen was frozen using the Colorado method (*Loomis* 1986) with one modification: before centrifugation, 0.25 ml of freezing solution was pipetted into the bottom of the tube instead of glucose-EDTA. After centrifugation for 15 min at 400 g, the spermatozoa had sunk into the freezing extender. The supernatant was aspirated and more freezing extender added, if necessary.

After 2 to 7 days of storage in liquid nitrogen, one straw per batch was thawed and the motility and concentration of spermatozoa checked. Post-thaw motility varied between 20% and 40%. Numbers of progressively motile spermatozoa/straw were between 200 and 400 x 10^6. One straw was thawed in a 37°C water bath for one min and used for insemination within 5 min of thawing. Ejaculates were used evenly within groups of mares and insemination times. Sixteen of the mares had foaled, 18 were maiden. Twenty-eight were Finnhorses and 6 warmblood riding horses. The mean ages of the foaling and maiden horses were 11.8 years (range 6-15 years) and 6.7 years (range 4-11 years), respectively. Mares with foals were inseminated during the second and third postpartum oestrus. Maiden mares were inseminated during 2 consecutive oestruses during the same months as the foaling mares, from May to July. All mares were inseminated during one oestrus, within 12 h postovulation and during one oestrus every 48 h before ovulation. The order of treatment was random. Blood samples were taken 3 times a week and serum progesterone determined by direct RIA (Spectria* Progesterone [^{125}I] Coated Tube Radioimmunoassay, Orion Diagnostica, Finland). Follicular status was monitored by rectal palpation and ultrasonography (Aloka SSP-210 DX 5MHz, Japan).

Table 1. Pregnancy rates of mares inseminated with frozen semen before and after ovulation.

Time of insemination before ovulation (h)	Number of mares pregnant/inseminated [pregnancy rate (%)]
< 12	2/8 (25)
12 - 24	4/9 (45)
Total < 24	6/17 (35)
24 - 36	3/10 (30)
36 - 48	1/7 (14)
Total 24 - 48	4/17 (23)
Total before ovulation	10/34 (30)
Total after ovulation	6/34 (18)

Examinations were started after the progesterone level had declined to 1 nmol/l. Pre-ovulatory insemination began when an oestrous mare had a follicle 35 mm in diameter. AI was repeated every other day until ovulation had been confirmed. All mares were examined every 12 h to determine the exact time of ovulation. AI was carried out using a bovine insemination rod (Cassou gun).

Examination for pregnancy was by ultrasound on the 15th day after ovulation. Pregnancies were terminated by intramuscular prostaglandin injection (Prosolvin, 7.5 mg. Intervet International B.V., 5830 AA Boxmeer, The Netherlands) at the same time, and the mares were inseminated again during the following heat.

Statistical analyses were carried out using the Chi-squared test and McNemar's test (*Woolson* 1987).

Results

Pregnancy rates in mares with foals (8/16) and maiden mares (8/18) were not statistically different ($p = 0.764$) when results relating to 2 oestrous cycles were combined. The pregnancy rate in mares < 10 yr of age (range 4-10 years) was 59% (13/22) and in mares > 10 yr of age (range 11-15 years) 25% (3/12) ($p = 0.057$). For mares inseminated < 24 h and 24-48 h before ovulation, pregnancy rates were 35% and 23% ($p = 0.4516$), respectively (Table 1). Lowest pregnancy rates were obtained with postovulatory insemination (18%) and with inseminations 36-48 h before ovulation (14%). The difference between combined pre-ovulatory (30%) and postovulatory (18%) AI was not significant ($p = 0.388$). The pregnancy rates for mares inseminated one, 2 or 3 times/oestrus were similar: 6/19 (31.5%), 3/12 (25%) and 1/3 (33.3%), respectively. On average, mares were inseminated 1.5 times/oestrus before ovulation.

Discussion

In the study reported here, overall pregnancy rates were lower than expected. It is not clear whether the semen quality of the stallion had declined over the years. Insemination times selected for this study did not produce satisfactory results with the semen used. The pregnancy rates might have been different if several stallions had been included. Because we wanted to compare timing of AI, only one stallion was, however, used.

In previous studies, AI every day or every other day before ovulation has given similar pregnancy rates: 30% and 31% (*Palmer & Magistrini* 1992), 55% and 55% (*Volkmann & van Zyl* 1987). Our results were similar: 35% within 24 h and 23% 24-48 h before ovulation. Pregnancy rates in mares inseminated > 36 h before ovulation have been low in all studies: 5% (*Pace & Sullivan* 1975), 20% (*Aliev* 1981), and 14% in this study. It is possible that the fertilizing capacity of frozen spermatozoa is not maintained for long. It is not known why frozen spermatozoa are unable to establish a reservoir of live spermatozoa in the area of the uterotubular junction or in the oviduct. Increasing the number of progressively motile spermatozoa in the AI dose above the critical level of approx. 200 x 10^6 (*Volkmann & van Zyl* 1987) does not seem to improve the results.

When the number of inseminations/cycle was greater than one, pregnancy rates were improved (Palmer & Magistrini 1992). No such improvement was noted in the study reported here but there were no adverse effects either. However the present study was based on few mares.

The postovulatory pregnancy rate was very low (18%) in our study. The percentage is, however, similar to that found by *Heiskanen et al.* (1994a) in an experiment in which mares were inseminated within 12 h postovulation in the same premises as the study reported here. Pregnancy rate/cycle was 14% higher in mares that had been examined and inseminated within 6 h after ovulation than in mares examined every 12 h (*Lindeberg & Kuntsi-Vaattovaara* 1992). Others have reported good results after AI within 6 h postovulation: 63% (*Martin et al.* 1979), 66% (Salazar-Valenzia 1983), 55% (*Kloppe et al.* 1988). These results compare favourably with those obtained following AI before ovulation (*Loomis et al.* 1983, *Cochran et al.* 1983, *Palmer* 1984, *Palmer & Magistrini* 1992). Although high pregnancy rates have been achieved when mares have been inseminated within 12 h after ovulation with fresh semen (*Koskinen et al.* 1990, *Woods et al.* 1990) and with fresh semen stored for 70 h or 80 h (*Heiskanen et al.* 1994b) such rates may not be achievable using frozen semen. Frozen-thawed spermatozoa may not be able to survive in a uterine environment changing from oestrogen-dominance towards increasing progesterone levels, or may not be capable of penetrating and fertilizing an older egg, the surface of which may have changed with age. The risk of early embryonic death also increases with time from ovulation (*Koskinen et al.* 1990, *Woods et al.* 1990).

The AI times used in this study - every other day before ovulation or within 12 h after ovulation - did not result in pregnancy rates that would be commercially acceptable. The times were probably too far from ovulation, at least for the semen used in the study. Payment policy, prices of individual semen doses, quality of semen and availability of veterinary services determine in practice which method of insemination is economically best when using frozen semen. If the price is per straw, only one insemination per oestrus can often be afforded. Mares have to be inseminated after confirmation of ovulation or hCG has to be administered to ensure ovulation within 36 h of AI. Use of hCG had no adverse effects on pregnancy rates when mares were inseminated with frozen semen (*Palmer & Magistrini* 1992). hCG is an excellent tool for managing timing of ovulation: numbers of palpations, inseminations and doses of frozen semen are reduced (*Love et al.* 1989). Not only stallions but mares should be selected. Older mares and mares with a history of endometritis or barrenness are not good candidates for insemination with frozen semen.

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Sammandrag

Inseminationstidpunktens inverkan på dräktighetsprocenten hos ston som inseminerats med fryst sperma.

Trettiofyra ston inseminerades med fryst sperma från samma hingst under 2 brunstcykler; varannan dag före ovulationen och inom 12 timmar efter ovulationen. Sperman frystes enligt Colorado-metoden. Inseminationsdosen var 200-400 x 10^6 progressivt motila spermier. Äggstockarna undersöktes var 12:e timme för att fastställa ovulationstidpunkten. Dräktighetsundersökning utfördes med ultraljud 15 dagar efter ovulationen. Trettiofem procent av de ston som inseminerats < 24 h och 23% av de ston som inseminerats 24-48 h före ovulationen var dräktiga ($p = 0.388$). Dräktighetsprocenten hos samtliga ston som inseminerats före ovulationen var 30% och hos ston som inseminerats inom 12 timmar efter ovulationen 18% ($p = 0.253$). Yngre ston (4-10 år) hade en högre dräktighetsprocent (59%) än äldre ston (11-15 år) (23%), skillnaden var dock inte statistiskt signifikant ($p = 0.057$).

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