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Reducing the examination interval to detect ovulation below 12 h does not improve pregnancy rates after postovulatory insemination with frozen/thawed semen in mares

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ABSTRACT

Data were analysed retrospectively from fourteen breeding seasons at an Equine Fertility Clinic for the effect of interval between pre- and postovulatory examinations for immediate postovulatory insemination on pregnancy rate (PR) and embryo loss rate (ELR). Mares of various breeds and ages were examined at intervals which varied from 0.5 to 15 h between the pre- and postovulatory period over 867 cycles. When ovulation was detected they were inseminated with a single dose of commercial frozen–thawed semen. All mares were treated in the post-insemination period with intrauterine antibiotics and then with oxytocin. Pregnancy diagnoses were made at 12–17 days post-ovulation and at intervals up to 40 days. The overall PR was 47.9%. The data were pooled into 3 h examination intervals. In the first interval, mares were inseminated at the time of ovulation to 3 h post-ovulation ($n = 44$) with a PR of 43.2%. Results of insemination to consecutive 3 h intervals gave PR of 44.7% (3–6 h, $n = 150$), 45.1% (6–9 h, $n = 432$), 55.8% (9–12 h, $n = 190$) and 54.9% (12–15 h, $n = 51$). ELR was 10.5%, 11.9%, 5.6%, 7.5% and 3.6% respectively for the same intervals. There was no statistical difference in either the PR or ELR. It is concluded that in a postovulatory insemination regime with routine post-insemination treatment as described, examination of mares at intervals of any less than 12–15 h does not improve pregnancy or embryo loss rates.

1. Introduction

It is generally accepted that fresh semen may normally remain capable of fertilisation in the mare for three or more days (Woods et al., 1990; Day, 1942; Newcombe, 1994, 2001). However, little is known of the longevity of frozen/thawed horse semen. The fertility of equine sperm may be seriously reduced by the process of freezing and thawing (Pickett et al., 1987; Brinsko and Varner, 1992; Pickett and Amann, 1993). Evidence suggests that although high quality frozen–thawed semen may be viable for up to 48 h in the mare's reproductive tract (Volkmann and Van Zyl, 1987), it is suspected that poorer samples may have a much more limited longevity.

Following ovulation, the viability of the oocyte to be fertilised satisfactorily is generally thought to be relatively short lived. In one study using fresh semen, embryo loss rate increased significantly in mares inseminated more than 6 h after ovulation (Woods et al., 1990). In contrast, Koskinen et al. (1990) reported that ova retained maximal viability for between 12 and 18 h after ovulation (examinations every 6 h). Timing of insemination with

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frozen/thawed semen therefore may be critical, not too early that the sperm die before the oocyte is ready for fertilisation, nor too late that the oocyte is over-mature.

When it is possible to accurately predict the timing of ovulation in the mare, then insemination at the point of ovulation would probably be the technique of choice. The use of human chorionic gonadotrophin (hCG) will determine the time of ovulation to within a 12-h interval (42 ± 6 h) in the majority of mares treated at a follicle diameter of 35 mm or larger (Barbacini et al., 2000). However, a minority of them will ovulate before hCG is given from follicles of $\leq$35 mm, while others will either ovulate too quickly after hCG administration (i.e. within 24 h), or fail to ovulate until more than 48 h later. Also occasionally a follicle will either regress or haemorrhage without collapse so that the oocyte is not released (Davies Morel and Newcombe, 2008). Since frozen horse semen is invariably expensive and often sold allowing only one or two insemination doses to establish a pregnancy, it can only be certain that semen is not wasted by waiting for a normal ovulation to occur.

If a postovulatory insemination regime is to be followed, then it is paramount to know for how long the oocyte remains viable. By examining mares either once or four times daily, high pregnancy rates (PRs) were achieved up to 24 h (Allen, 1981) and 18 h (Koskinen et al., 1990) after ovulation, respectively, using fresh semen. Kedrov (1945) reported a PR of >75% at 2, 4, 6, 8, and 10 h post-ovulation ($n = 105$) compared with 64% 0–24 h before ovulation. A recent report (Sheerin et al., 2001) found a non-significant difference following insemination in a small group of mares with frozen/thawed semen up to 12 h after ovulation compared with up to 12 h before. Similarly, Sieme et al. (2003) obtained similar results with frozen/thawed semen up to 12 h after ovulation (50% of 48) that up to 12 h before ovulation (41.3% of 75). It therefore may be more logical to have the oocyte waiting in the fallopian tube for the sperm than the sperm waiting for ovulation, which is normal practice with natural mating or with fresh/chilled semen.

A 12-h interval examination protocol is much less time demanding for the veterinarian compared with an every-6 h interval examination which is common practice amongst equine practitioners. Perhaps this old practice still is maintained owing to the results of the study of Woods et al. (1990) that showed that the PR falls and the embryo loss rate (ELR) rises with increasing interval between ovulation and insemination (starting 6 h post-ovulation). The previous study is from the 90s in which fresh semen was used while post-mating treatment was not given after AI to mitigate the post-mating uterine reaction.

The aim of this study was to determine the optimum time interval between the pre- and the post-ovulation examinations on the PR in a large number of mares inseminated with frozen/thawed semen and administered a routine post-mating treatment.

2. Materials and methods

2.1. Mares

Data were analysed from fourteen breeding seasons (1996–2009) for the effect of the interval between examinations, for immediate postovulatory insemination on PR and ELR following AI in 867 cycles in over 700 mares.

Mares of various breeds aged 2–23 years were resident at the clinic during oestrus and were examined by palpation and with ultrasound, using a 5–7 MHz transrectal linear-array transducer at intervals initially of 48–72 h. As ovulation was thought to be approaching, the interval between examinations was reduced to 24 h, then to 12 h, and finally 8 h so that ovulation should not occur within an examination interval of much more than 8 h. On some occasions, when ovulation appeared imminent, the interval was reduced to less than 8 h. Human chorionic gonadotrophin (hCG) was not routinely used to advance ovulation. Upon detection of ovulation, mares were inseminated without undue delay. No mare was inseminated with more than a single dose of semen at any one oestrous period.

2.2. Frozen spermatozoa and insemination protocol

Semen used was from over 250 stallions of many horse breeds frozen in the UK, the USA and various European countries. Semen was thawed at 37° for at least 30 s, transferred into a narrow lumen catheter and inseminated deeply into the uterine horn ipsilateral to the ovulation. The quality of the semen varied from very poor to excellent (Newcombe, 1999). Although the post-thaw progressive motility of spermatozoa was not taken into account for the data analysis, it is assumed that the allocation of a given spermatozoa sample to a mare at a given examination interval was random, since the frequency of examinations depended on staff availability and not on post-thawed spermatozoa progressive motility.

2.3. Post-insemination treatment

Because in our experience and that of others (Muller, 1987) there appears to be a higher degree of post-mating induced endometritis observed following frozen/thawed insemination, all mares were routinely treated after insemination. The post-mating treatment consisted of an intrauterine infusion of 12 ml of a mixture of injectable procaine penicillin (1800 mg) suspension (6 ml Depocillin®) and 900 mg framycetin (6 ml Framomycin® 15% injection; C-Vet) 8 h post-insemination followed by a bolus of 25 IU oxytocin (Oxytocin Leo; Leo Laboratories) administered intravenously at the next examination 8–12 h later. If ultrasonic evidence of fluid was still present at subsequent examinations, treatment with oxytocin was repeated. Pregnancy diagnoses were made initially between 12 and 17 days after insemination and again at day 30 or 40 post-insemination. Any pregnancy loss up to near term reported in a mare that had not been re-examined at the 30–40 day interval, was included in the data as if it were an embryonic loss.

2.4. Experimental design

The interval between pre- and postovulatory examinations was calculated from the examination in which the preovulatory follicle was still visible to the point when insemination was performed (at the time when ovulation