Effect of repeated transvaginal ultrasound-guided follicle aspiration on fertility in mares

Dirk K. Vanderwall, DVM, PhD, DACT; Kevin J. Hyde, DVM; Gordon L. Woods, DVM, PhD, DACT

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Objective—To determine whether performance of transvaginal ultrasound-guided follicle aspiration (TVUFA) repeatedly in mares adversely affects their fertility.

Design—Historical prospective study.

Animals—23 mares that had never undergone TVUFA and 59 mares that had undergone TVUFA on 1 to 11 occasions.

Procedure—Mares were classified into 4 groups according to the number of TVUFA procedures previously performed on the ovary in which ovulation occurred at the time of insemination as follows: group 1, 0 TVUFAs (control group, n = 23 mares); group 2, 1 or 2 TVUFAs (40 mare-cycles); group 3, 3 or 4 TVUFAs (21 mare-cycles); and group 4, 5 to 11 TVUFAs (13 mare-cycles). Each ovary and its associated number of TVUFAs were considered separately; therefore, some of the mares that underwent TVUFA were represented in > 1 group (1 mare was included in group 2 twice [once for each ovary]), and the sample size in groups 2, 3, and 4 was denoted as mare-cycles. Fertility was assessed as pregnancy rates in cycles in which mares were inseminated with fresh or cooled semen from 1 fertile stallion.

Results—There were no significant differences in pregnancy rates among groups 1, 2, 3, and 4 (83%, 90%, 81%, and 85%, respectively).

Conclusions and Clinical Relevance—Results indicated that repeated performance of TVUFA (as many as 11 times) had no detectable adverse effect on fertility in mares. This finding is clinically important for situations when TVUFA is performed on fertile mares, whether for oocyte collection or other purposes. (*J Am Vet Med Assoc* 2006;228:248–250)

Transvaginal ultrasound-guided follicle aspiration is being used increasingly to recover oocytes from mares that are fertile or clinically subfertile (classified as such on the basis of various reproductive problems). In clinically subfertile mares, TVUFA is used for oocyte transfer,[1] which involves collection of 1 or more oocytes from a subfertile mare and transference of an oocyte to the oviduct of an inseminated recipient mare. In fertile mares, TVUFA is used to obtain oocytes for applications such as in vitro oocyte maturation,[2] intracytoplasmic injection of spermatozoa,[3] and cloning.[4] In addition to its use for collecting oocytes, TVUFA is performed experimentally to collect follicular fluid,[5] administer intrafollicular treatments,[6] or ablate follicles[7] as part of investigations to characterize the factors that regulate follicular growth and ovulation in mares.

Despite being minimally invasive, repetitive performance of TVUFA in mares causes fibrosis in the ovarian stroma[8]; although such fibrosis does not appear to interfere with ovarian function,[8] its development illustrates the potential for TVUFA procedures to have adverse effects. For example, because of its proximity to the ovary, it is plausible that the function of the oviduct in a mare could be compromised as a result of a TVUFA procedure; whether such a potentially deleterious effect occurs in mares and whether it has an impact on fertility subsequently are unknown. In sheep, repetitive follicular puncture performed endoscopically resulted in the development of adhesions between the ovary and oviduct in 6 of 20 (30%) ewes, and although the adhesions did not appear to impair fertility, it further exemplifies the potential for repetitive ovarian or follicular puncture to have deleterious effects.[9] For subfertile mares, it is relatively inconsequential whether the TVUFA procedure itself is detrimental to fertility because preexisting reproductive problems are the underlying reason for performing TVUFA (eg, for oocyte transfer). In contrast, when performing TVUFA on fertile mares, it is important to know whether the procedure itself subsequently alters fertility because any loss of fertility would be an extremely undesirable consequence. The objective of the study reported here was to determine whether performance of TVUFA repeatedly in mares adversely affects their fertility.

Materials and Methods

Data collection—Reproductive records from 1999 to 2004 for research mares at the University of Idaho Northwest Equine Reproduction Laboratory were reviewed to obtain all of the data for this historical prospective study. Mares were of mixed breeding; they were 3 to 12 years old and weighed 300 to 500 kg (660 to 1,100 lb). Pregnancy rates of 23 mares that had never undergone TVUFA and 59 mares in which TVUFA had been performed 1 to 11 times prior to insemination were compared.

For mares that had previously undergone TVUFA, the procedure had been performed to recover in vivo matured oocytes for cloning.[10,11] The TVUFA procedure was performed

TVUFA Transvaginal ultrasound-guided follicle aspiration

From the Northwest Equine Reproduction Laboratory, Department of Animal and Veterinary Science and Center for Reproductive Biology, College of Agricultural and Life Sciences, University of Idaho, Moscow, ID 83844-2201. Dr. Hyde's present address is Rood and Riddle Equine Hospital, PO Box 12070, Lexington, KY 40580.

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Address correspondence to Dr. Vanderwall.

with mares restrained in stocks. After evacuation of fecal matter from the rectum, the perineal area was cleansed with soap and water. Just prior to the TVUFA procedure, mares were administered xylazine hydrochloride (0.6 mg/kg [0.27 mg/lb], IV), acepromazine maleate (0.03 mg/kg [0.014 mg/lb], IV), and butorphanol tartrate (0.01 mg/kg [0.005 mg/lb], IV). In addition, they received propantheline bromide[a] (0.1 mg/kg [0.045 mg/lb], IV) to induce relaxation of the rectum. Transvaginal ultrasound-guided follicle aspiration was then performed by use of a 6.5-MHz curvilinear transducer[b] with an external needle guide equipped with a 60-cm, 12-gauge double-lumen ovum pick-up needle.[c] The ultrasound transducer was introduced into the fornix of the vagina while the ovary bearing the follicle or follicles to be aspirated was manipulated per rectum. The follicle was aspirated by positioning the ovary against the face of the transducer (with the needle guide as a reference) and then advancing the needle into the follicle. After puncturing the follicle, the contents of the follicle were aspirated by use of a vacuum pump maintained at –150 mm Hg. As the follicular fluid was being aspirated, the follicle was massaged per rectum and was irrigated with approximately 150 mL of modified Dulbecco phosphate-buffered saline solution with 1% (vol/vol) fetal bovine serum, heparin (10 U/mL), penicillin (100 U/mL), and streptomycin (100 µg/mL). Only mature preovulatory follicles were aspirated; therefore, in general, 1 follicle was punctured in an ovary during each TVUFA procedure. When mares developed multiple (2 or 3) preovulatory follicles in 1 ovary (approx 17% of TVUFA procedures), each follicle was punctured separately; for the purposes of this study, only 1 TVUFA procedure for that ovary was recorded. Each ovary (and its associated number of TVUFA procedures) was considered separately. Mares were classified into 4 groups according to the number of TVUFA procedures previously performed on the ovary in which ovulation occurred when they were inseminated: group 1, 0 TVUFAs (control, n = 23 mares); group 2, 1 or 2 TVUFAs (40 mare-cycles); group 3, 3 or 4 TVUFAs (21 mare-cycles); and group 4, 5 to 11 TVUFAs (13 mare-cycles). Because each ovary and its associated number of TVUFA procedures were considered separately, some of the 59 mares that underwent TVUFA were represented in > 1 group, and 1 mare was included in group 2 twice (once for each ovary); therefore, the sample sizes in groups 2, 3, and 4 were denoted as mare-cycles.

Breeding management and pregnancy diagnosis— Fertility was assessed during the cycles in which mares were inseminated with fresh or cooled semen from 1 fertile stallion (same stallion for all mares). The reproductive tracts of mares were monitored 4 times/wk via transrectal palpation and ultrasonography to assess ovarian follicular activity and the degree of endometrial edema. When mares developed an ovarian follicle ≥ 30 mm in diameter that was accompanied by prominent endometrial edema, they were evaluated daily or every other day until ovulation was detected (day 0). Ovulation was defined as disappearance of follicles > 35 mm in diameter between 2 successive examinations, accompanied by ultrasonographic evidence of a corpus luteum. When the dominant follicle became ≥ 35 mm in diameter, mares were treated with human chorionic gonadotropin (5 U/kg [2.3 U/lb], IV). Insemination was performed either at the time that human chorionic gonadotropin was administered (by use of fresh semen) or approximately 24 hours after human chorionic gonadotropin was administered (by use of cooled semen). Semen was collected from the stallion by use of an artificial vagina and then evaluated for gel-free volume, concentration, and progressive motility of spermatozoa by use of standard procedures. Each insemination dose of fresh semen contained at least 500 million progressively motile spermatozoa; each dose was mixed 1:1 with skimmed milk glucose extender,[d] after which it was stored (< 1 hour) at room temperature (24°C [75°F]) until insemination (used for insemination during 82 mare ovulatory cycles). Each insemination dose of cooled semen contained at least 1 billion progressively motile spermatozoa (determined before cooling); each dose was mixed with skimmed milk glucose extender[e] to a final concentration of 25 to 50 million spermatozoa/mL, after which it was stored (approx 24 hours) in a passive cooling unit[f] until insemination (used for insemination during 15 mare ovulatory cycles).

Pregnancy status was determined via embryo recovery on days 6 to 8 (n = 8 mare-cycles) or transrectal ultrasonography on days 12, 13, and 14 (89 mare-cycles) after ovulation. Ultrasonographic diagnosis of pregnancy in a mare required identification of an embryonic vesicle that changed location within the uterine lumen or increased in diameter during the interval between 2 consecutive daily examinations. Mares with unilateral multiple ovulations were considered pregnant if at least 1 conceptus was identified. Data from mares with bilateral multiple ovulations were included only if the pregnancy status of all ovulations could be confirmed.

Statistical analysis— Statistical analyses were performed with computer software.[g] Pregnancy rates were compared among groups 1 to 4 via χ^2 analysis. A value of $P < 0.05$ was considered significant.

Results

There was no significant ($P > 0.10$) difference in pregnancy rate between mares inseminated with fresh semen (87.5%) and those inseminated with cooled semen (86.7%); therefore, the type of semen used did not bias the outcome of the study. Similarly, there was no significant ($P > 0.10$) difference in pregnancy rate between mares that underwent embryo recovery (100%) and those that underwent ultrasonography for pregnancy diagnosis (84.3%); therefore, the method of pregnancy diagnosis did not bias the outcome of the study.

In group 1 mares (those that had never undergone TVUFA before), the pregnancy rate was 83%. In mares that had undergone TVUFA on 1 or 2 occasions, the pregnancy rate was 90%. In mares that had undergone TVUFA on 3 or 4 occasions, the pregnancy rate was 81%. In mares that had undergone TVUFA on 5 to 11 occasions, the pregnancy rate was 85%. There were no significant ($P > 0.10$) differences in pregnancy rates among groups.

Discussion

The results of the study reported here indicated that repeated performance of TVUFA (as many as 11 times) in mares had no detectable adverse effect on their subsequent fertility; there were no differences in pregnancy rates between control mares that had never undergone TVUFA and mares that had undergone TVUFA previously. This finding is clinically important for situations when TVUFA is performed on fertile mares, whether for oocyte collection or other purposes, because it highlights that TVUFA can be performed (as described in our study) without compromising a mare's future fertility. Although it was not possible to completely rule out the possibility that fertility may have been adversely affected in an individual mare, there was no evidence of a widespread adverse effect of the TVUFA procedure on fertility via analysis of the groups' data.

In a report[8] on the effect of repetitive follicular puncture in 4 mares, Bøgh et al determined that ovarian

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function (defined as the ability to regularly ovulate preovulatory follicles and develop corpora lutea) remained apparently normal after several years of use for TVUFA, during which time each mare's ovaries had been punctured approximately 122 times (ie, each ovary punctured a mean of 61 times). Although ovarian function was not affected, pathologic changes were detected when the ovaries were examined after ovariectomy or during necropsy. All of the ovaries had increased fibrosis in the ovarian stroma, compared with ovaries from control mares that had not undergone TVUFA. In addition, in that study,[8] the left ovary of 1 mare that underwent TVUFA multiple times developed an adhesion to the abdominal wall and spleen and contained several small abscesses within the ovarian stroma, whereas the mare's right ovary contained a 3 × 2-cm cystic structure that was surrounded by a cartilaginous capsule and contained yellow-brown mucus. In the present study, there was no evidence that adhesions had developed between an ovary and abdominal viscera or abdominal wall (eg, decreased mobility of the ovary on palpation) in any of the mares; however, the development of adhesions between an ovary and surrounding reproductive tissues such as the oviduct could not be ruled out.

It is important to note that when the mares in the present study underwent TVUFA, it was to aspirate a single preovulatory follicle in an ovary (unless there were multiple preovulatory follicles in the same ovary); therefore, the number of times that TVUFA was performed closely corresponded to the number of times that an individual ovary was punctured. In contrast, when all follicles (> 5 to 8 mm) are aspirated, each ovary is punctured approximately 6 to 7 times during a single TVUFA session[12,13]; in this situation, the number of punctures of an ovary is generally greater than that associated with TVUFAs performed repeatedly to collect only preovulatory follicles. Because the total number of times an ovary is punctured may directly influence the development of deleterious effects, the results of our study should not be extrapolated to situations in which mares' ovaries are routinely punctured numerous times during each TVUFA session. Further work will be needed to determine whether fertility in mares is adversely affected when multiple ovarian punctures are routinely made during each TVUFA session. In addition to the number of times the ovary is punctured, there are other aspects of the TVUFA procedure (eg, needle gauge, vacuum pressure, and degree of ovarian or follicular manipulation during aspiration) that could be modified from conditions used in the present study. Whether such a change in one or more of those factors would alter the likelihood of the TVUFA procedure resulting in adverse effects on fertility in mares is not known. On the basis of the data collected after TVUFA performed in the manner used in our study, repeated TVUFA procedures in mares do not appear to subsequently compromise their fertility.

a. Monument Pharmacy, Monument, Colo.
b. SonoVet 600, Universal Medical Systems Inc, Bedford Hills, NY.
c. V-EOAD-1260-L, Cook Veterinary Products, Spencer, Ind.
d. EZ-Mixin OF, Animal Reproduction Systems, Chino, Calif.
e. EZ-Mixin CST, Animal Reproduction Systems, Chino, Calif.
f. Hamilton Research Inc, South Hamilton, Mass.
g. SAS, version 8.01, SAS Institute Inc, Cary, NC.

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