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

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, 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, intracytoplasmic injection of spermatozoa, and cloning. In addition to its use for collecting oocytes, TVUFA is performed experimentally to collect follicular fluid, administer intrafollicular treatments, or ablate follicles 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; although such fibrosis does not appear to interfere with ovarian function, 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. 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. The TVUFA procedure was performed...
with mares restrained in stocks. After evacuation of fecal mat-
er 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 (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 with
an external needle guide equipped with a 60-cm, 12-gauge
double-lumen ovum pick-up needle. The ultrasound trans-
ducer 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 posi-
tioning the ovary against the face of the transducer (with the
manipulating hand as a reference) and then advancing the
needle as a reference) and then advancing the needle
to the follicle. After puncturing the follicle, the contents of
the follicle were aspirated by use of a vacuum pump main-
tained at −130 mm Hg. As the follicular fluid was being aspir-
ated, 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 aspirat-
ed; therefore, in general, 1 follicle was punctured in an ovary
during each TVUFA procedure. When mares developed mul-
tiple (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 num-
er 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 TVUFA (control, n = 23 mares); group 2, 1 or 2 TVUFA
(40 mare-cycles); group 3, 3 or 4 TVUFA (21 mare-cycles);
and group 4, 5 to 11 TVUFA (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
every other day until ovulation was detected (day 0). Ovulation
was defined as disappearance of follicles > 35 mm in
length between 2 successive examinations, accompa-
nied 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,4 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 bil-
lion progressively motile spermatozoa (determined before
cooling); each dose was mixed with skimmed milk glucose
extender to a final concentration of 25 to 50 million sper-
matozoa/mL, after which it was stored (approx 24 hours) in
a passive cooling unit until insemination (used for insemi-
nation during 15 mare ovulatory cycles).

Pregnancy status was determined via embryo recovery
on days 6 to 8 (n = 8 mare-cycles) or transrectal ultrasonog-
raphy 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 ovoidations were considered pregnant
if at least 1 conceptus was identified. Data from mares with
bilateral multiple ovoidations were included only if the preg-
nancy status of all ovoidations could be confirmed.

Statistical analysis—Statistical analyses were performed
with computer software.1 Pregnancy rates were compared
among groups 1 to 4 via χ² 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 pregnan-
cy 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 preg-
nancy 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 sub-
sequent fertility; there were no differences in pregnancy
rates between control mares that had never undergone
TVUFA and mares that had undergone TVUFA previ-
ously. This finding is clinically important for situations
when TVUFA is performed on fertile mares, whether for
oocyte collection or other purposes, because it high-
lights 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 affect-
ed 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 report7 on the effect of repetitive follicular
puncture in 4 mares, Bagh et al determined that ovarian

JAVMA, Vol 228, No. 2, January 15, 2006
Scientific Reports: Original Study 249

Unauthenticated | Downloaded 12/23/23 07:36 PM UTC
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, 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; 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.

References