Effects of Preovulatory Tranquilization with Xylazine on the Timing of Ovulation in the Horse

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Xylazine is the most commonly used equine tranquilizer but its effects on ovulation and periovulatory endocrinology have not been tested in the mare. In this study, mares received xylazine (0.5 mg/kg IV) or vehicle during synchronized follicular development. The diameter of the dominant ovarian follicle was monitored every twelve hours by ultrasonography and the time of ovulation was recorded. Serum concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH) and progesterone were measured by radioimmunoassay. Xylazine transiently but significantly increased concentrations of FSH. LH and progesterone profiles as well as follicular diameters and the timing of ovulation were similar following treatment with xylazine or vehicle. A single tranquilizing dose of xylazine is unlikely to alter ovulation in the mare.

Key words: FSH, horse, ovulation, tranquilization, xylazine

Tranquilization of the mare is a common procedure used to facilitate transport and handling prior to breeding. Alpha-2 agonists such as xylazine (XYL) are used commonly for this purpose, alone or in combination with other forms of chemical restraint [3, 27]. Unfortunately, this need for tranquilization of the broodmare coincides with stages of follicular development and ovulation that are delayed by tranquilizers and anesthetic agents in other species (Table 1). Tranquilizers that interfere with ovulation compromise fertility by preventing timely delivery of the ovum into the reproductive tract for fertilization [29].

In the current study, the impact of preovulatory treatment with a single tranquilizing dose of xylazine on the timing of ovulation and periovulatory endocrinology was examined in the mare.

Materials and Methods

Animal Treatments

All procedures were reviewed by the institutional animal care and use committee of the University of Kansas Medical Center. Adult quarter horse and thoroughbred mares (4–8 years of age, 400–500 kg) were housed in individual stalls and provided with water and mixed hay ad libitum and given concentrate twice daily. Experiments were performed during July and August in the northern hemisphere. Normally cycling mares (n=6 mares, n=12 cycles) received xylazine or vehicle during the preovulatory interval (Fig. 1). Each was designated to receive xylazine or vehicle in random order during two separate cycles. One recovery cycle was allowed to elapse between experimental cycles. Following informed consent, synchronized luteolysis and follicular development were induced by administration of prostaglandin F2α.
(PGF, 12.5 mg IM; Upjohn, Kalamazoo, MI, USA). Two xylazine and one control cycle were excluded from two different mares due to a failure of synchronization. Follicular development and uterine tone were monitored by palpation per rectum and ultrasonography (Pie Data 480; 5 MHz probe; Maastricht, Netherlands) every 12 or 24 hr beginning at 72 hr post-PGF and continuing until confirmation of ovulation. The diameter of the largest ovarian follicle was recorded at each observation. Blood samples were collected prior to each palpation for subsequent analysis of progesterone and gonadotropin concentrations. Tranquilization with xylazine (0.5 mg/kg IV) or administration of saline vehicle was performed once the dominant ovarian follicle (F1) had developed to 2.5 cm in diameter in each animal. Growth of F1 to a diameter of 2.5–3 cm was coincident with the onset of the preovulatory rise in luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the mare in previous studies [8, 15]. We timed treatments from this objective parameter of follicular development (i.e. follicular diameter) rather than the onset of behavioral estrus to avoid difficulties from silent estrus. In a preliminary study, high ambient temperature contributed to increased silent estrus although follicular development and ovulation appeared normal. The interval just prior to and during rising serum FSH and LH is when other species are most sensitive to blockade of ovulation by tranquilizers and anesthetics (Table 1, [5, 21]).

**Hormonal analyses**

Concentrations of equine LH, FSH and progesterone in sera were measured by specific radioimmunoassays. Briefly, LH and FSH were measured in duplicate from 100 µl aliquots using a previously validated dual antibody method [6]. Antibodies for equine FSH and LH were AFP-2062096 and AFP-240580. Highly purified FSH (AFP-5022B) and LH (AFP-5130A) were used for radiiodination and the reference standard. Progesterone was measured in duplicate from 20 µl aliquots as described previously [30, 40]. Intra- and interassay coefficients of variations were less than 10% for all assays.

**Statistical Analyses**

Data were first compared for an effect of xylazine on the timing of ovulation using a Student’s t-test. Time of ovulation was expressed relative to PGF administration or time of xylazine/vehicle administration (which coincided with follicular development to 2.5 cm in diameter). Upon finding no effect of xylazine on the timing of ovulation, follicular diameters and hormone concentrations (LH, FSH, progesterone) were expressed relative to ovulation and analyzed using an ANOVA for repeated measures with time and treatment (i.e. xylazine) as main effects. When main effects or interaction terms were significant (p<0.05),
individual means were compared using a Tukey’s test. Data from three cycles (two xylazine and one control cycle) were not collected due to failure to respond to PGF with subsequent follicular growth. Statistical differences were considered significant at $p<0.05$ throughout the analysis.

### Results

#### Synchronization of follicular development

Nonpregnant mares in the luteal phase (moderate uterine tone and no ovarian follicles $>2$ cm in diameter) received PGF (12.5 mg IM) and were checked for response (i.e., follicular development) beginning 48 hr later. Synchronized cycles were characterized by progressive follicular development. Mares received xylazine tranquilization or saline vehicle once the largest ovarian follicle had reached a diameter of 2.5 cm (Table 2). Previous studies have shown that this stage of follicular development immediately precedes the preovulatory rise of FSH and LH in the mare [13]. All mares receiving xylazine were tranquilized (20–30 min) as indicated by decreased responsiveness to stimuli, mild ataxia and incoordination.

#### Effects of xylazine on ovulation

Ovulation during control cycles occurred approximately 9 days after PGF and 3.9 days post-treatment (saline, Table 2). This is in agreement with previous studies examining ovulation after treatment with PGF [22]. Xylazine had no effect on the timing of ovulation. This was true whether the timing of ovulation was expressed as days from PGF or related to the day of treatment (2.5 cm diameter). Similarly, follicular diameter at ovulation did not differ with xylazine treatment ($p>0.30$).

#### Xylazine and ovulation

<table>
<thead>
<tr>
<th>Trt</th>
<th>Time (Days post-PGF)</th>
<th>F1 dia (cm) at trt</th>
<th>Ovulation (Days post-PGF)</th>
<th>Ovulation (Days post-trt) at Ovulation</th>
<th>F1 dia (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL ($n=5$)</td>
<td>5.0 ± 0.69</td>
<td>2.5 ± 0</td>
<td>9.2 ± 0.61</td>
<td>3.90 ± 0.43</td>
<td>3.55 ± 0.24</td>
</tr>
<tr>
<td>XYL AZINE ($n=4$)</td>
<td>5.0 ± 1.08</td>
<td>2.55 ± 0.05</td>
<td>8.5 ± 1.48</td>
<td>3.50 ± 0.18</td>
<td>3.88 ± 0.38</td>
</tr>
</tbody>
</table>

Mares received xylazine (0.5 mg/kg IV) or saline vehicle once the largest ovarian follicle reached 2.5 cm in diameter. Xylazine failed to alter the timing of ovulation or follicular size at ovulation. Dia=diameter, F1=largest ovarian follicle, trt=treatment. There were no significant differences ($p>0.30$) between control and xylazine-treated groups.

#### Xylazine and follicular development

Xylazine had no effect ($p=0.34$, Fig. 2) on the trajectory of follicular development as assessed by the diameter of the largest ovarian follicle (F1). Following treatment with vehicle or xylazine once the F1 reached 2.5 cm in diameter, growth of the F1 continued until ovulation approximately 4 days later. This progressive follicular growth was reflected as a significant effect of time on follicular diameter ($p=0.006$) in the statistical analysis.

#### Xylazine and hormone profiles

Hormone concentrations for the gonadotropins, LH and FSH, in addition to progesterone, an indicator of luteal function, were measured every 12 hr following treatment with xylazine or saline vehicle until ovulation (Fig. 3. A, B, C). Mares receiving saline vehicle exhibited progressively greater concentrations of serum LH as ovulation approached. In contrast, concentrations of serum LH changed less as ovulation approached following xylazine administration, although there was also a preovulatory rise in LH in these animals. Nonetheless, LH profiles did not differ

![Fig. 2. Diameter of the largest ovarian follicle (F1) during the period of final maturation and ovulation ($n=5$ control, $n=4$ xylazine).](image-url)
significantly between controls and xylazine-treated mares \((p=0.34, \text{Fig. 3. A})\). FSH was the only hormone altered by xylazine although there was a significant effect of time for all hormones examined. Serum concentration of FSH was significantly higher \((p=0.008)\) in xylazine-treated mares at 3.5 days prior to ovulation (immediately following treatment, Fig. 3. B). Concentrations of serum FSH were similar in both groups for the remainder of the preovulatory period. Progesterone concentrations were high but variable for both groups at the time of PGF administration \((4.1 \pm 1.2 \text{ ng/ml} \text{ in the control group and } 2.9 \pm 0.8 \text{ ng/ml} \text{ in the xylazine-treated group, data not shown})\) but had declined to consistently low concentrations \(<0.5 \text{ ng/ml}\) by 3.5 days prior to ovulation (Fig. 3. C). This reflects the loss of luteal function following PGF action. Progesterone remained low until immediately prior to ovulation when concentrations rose significantly in both groups.

**Discussion**

Xylazine is widely used for tranquilization of horses including broodmares. Since preovulatory tranquilization or anesthesia with other drugs delays or prevents ovulation in many species (Table 1) and xylazine alters gonadotropin release in anestrous mares [12], it is important to test for effect of preovulatory xylazine administration on ovarian function and ovulation. The current study found no significant effect of tranquilization with a single dose of xylazine \((0.5 \text{ mg/kg IV})\) on the timing of ovulation. It is unlikely that xylazine, as administered near the onset of final follicular development in a tranquilizing (i.e. not anesthetic) dose in the current study, alters ovarian function.

One challenge of the current study was choosing the time of treatment. In other species, tranquilization just prior to the onset of the preovulatory LH/FSH surge (which generally occurs on the day of ovulation) is most likely to delay or prevent ovulation [20, 21]. Ovulation in the mare is somewhat unique, however, because the preovulatory rise in LH and FSH begins 2–4 days prior to ovulation rather than immediately preceding it [10, 23, 25, 26, 36, 37]. We reasoned that the neural mechanisms immediately preceding and reinforcing the elevation in FSH and LH would be the aspect of ovulatory physiology most sensitive to tranquilization (as is the case in other species, Table 1). We therefore opted to test for effects of xylazine during the interval of rising FSH and LH (with negative results) rather than focusing on the day of ovulation itself. This treatment period also coincides with the initial emergence of the dominant follicle in the mare [8, 15].

Both our data and the work of Fitzgerald and Mellbye (anestrous mares [12]) showed a tendency for xylazine to elevate gonadotropin concentrations although this effect was only significant for FSH in the current study. Nevertheless, because xylazine has shown no inhibitory effect on gonadotropin release in either study, it seems unlikely that xylazine would retard follicular development and ovulation regardless
of the timing of administration. Furthermore, the uniquely prolonged elevation of LH and FSH preceding ovulation in the mare may result in an ovulatory mechanism that is less sensitive to transient alterations in gonadotropins than other species. In summary, a single tranquilizing dose of xylazine is safe for use in the mare prior to breeding.

Acknowledgments

The authors thank the owners, staff and animals of Settler’s Acre Farm, Greenwood, MO, USA, for their assistance during this study. NIDDK RIA kits for equine FSH and LH were kindly provided by Dr. A. F. Parlow. This work was supported in part by Grant-in-Aid from the Equine Research Institute of the Japan Racing Association, and a Grant-in-Aid for Scientific Research (The 21st Century Center of Excellence Program, E-1) from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan.

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