1. Introduction
The recent acceptance of frozen semen by major breed registries has stimulated the increased use of frozen semen for insemination of mares.\(^1\) In addition, techniques have improved for freezing and thawing, which have provided higher fertility than previously reported.\(^1\)–\(^3\) Furthermore, fixed-time insemination protocols allow the mare inseminated with frozen semen to be examined only one time per day during estrus, similar to those bred with cooled semen.\(^4\) It has generally been stated that frozen semen should not be used for insemination of barren mares.\(^5\) However, limited data are available on comparison of fertility of barren mares bred with fresh versus frozen-thawed semen. Additionally, factors affecting the fertility of barren mares bred with frozen-thawed semen generally have not been examined. The objectives of this study were to determine fertility of barren mares inseminated with either fresh or frozen-thawed semen, to evaluate the effects of mare age, uterine fluid, and number of inseminations of mares bred with frozen-thawed semen, and to evaluate the effect of age on response to hCG for barren mares inseminated with frozen-thawed semen.

2. Materials and Methods
During the breeding seasons of 1998–2007, 285 barren mares of various breeds (90% Warmbloods or Quarter Horse types) from 3 to 21 yr of age were inseminated with frozen-thawed semen. Number of years barren ranged from 1 to 3. The majority of mares were cultured, biopsied, and treated during the fall before the breeding season during which they were inseminated. Semen of good quality and frozen in many different laboratories (46 stallions) was used. Frozen semen had at least 30% post-thaw motility and at least 200 million motile sperm per dose. Of the mares, 238 were inseminated one time per cycle within a 6-h pre-ovulation and a 6-h post-ovulation period; 59 were inseminated two times (24 and 40 h after hCG injection). Data from other mares were available during the 2006–2007 season.
breeding season. This included 133 Standardbred barren mares inseminated with fresh semen, 4–19 yr of age. For fresh inseminations, mares and stallions were at the same farm, and mares were inseminated with good-quality fresh semen (500 million motile sperm per dose) from stallions of proven fertility. All mares were artificially inseminated in the uterine body. Data were analyzed by \( \chi^2 \)-square analysis, and the level of significance was \( p < 0.05 \).

3. Results and Discussion

The 285 mares inseminated with frozen-thawed semen were inseminated for a total of 544 cycles. This resulted in 202 pregnancies (75/202 [37%]; overall seasonal pregnancy rate of 71% [143/202]). The per-cycle pregnancy rate for mares 10–16 yr of age (41%) was similar to those 3–9 yr of age (38%) but more than those 16 yr of age (23%). Overall age (41%) was similar to those 3–9 yr of age (38%). The per-cycle pregnancy rate for mares 10–16 yr of age had a higher (\( p < 0.05 \)) pregnancy rate than mares <16 yr of age. Mares >16 yr of age had a higher percentage of ovulations outside the window of 24–48 h compared with mares in the other two age groups. Sixteen percent of cycles (71/445) had ovulations >48 h, but the means were similar for age groups.

Fluid accumulation in the uterus was examined at 18–24 h post-artificial insemination. Any mare having >20 mm diameter of fluid in the uterus was considered to have fluid accumulation. Those in that category were treated with 20 IU, IV, of oxytocin and/or uterine lavage. Surprisingly, mares that had fluid in the uterus post-artificial insemination resulted in a higher (\( p < 0.05 \)) per cycle pregnancy rate than those without fluid (52% vs. 29%, respectively). This apparently was caused by the positive effect of oxytocin and/or lavage treatment in this group of mares. When the interaction of age and fluid accumulation post-artificial insemination was examined, only mares in the category of 10–16 yr of age had a higher (\( p < 0.05 \)) pregnancy rate if fluid accumulation was detected (66% vs. 28% per cycle pregnancy rate). There was a significantly higher per cycle pregnancy rate and overall pregnancy rate for mares inseminated on a fixed-time insemination (24 and 40 h after hCG versus those inseminated only one time in the cycle): 65% vs. 83% and 34% vs. 60% (\( p < 0.05 \)), respectively. One could not statistically compare fertility of barren mares bred with fresh semen to those bred with frozen-thawed semen because of the different populations of mares; per cycle pregnancy rates and overall pregnancy rates for mares inseminated with frozen and fresh semen were 37% vs. 34% and 71% vs. 77%, respectively. However, the means were nearly identical.

Based on these studies, insemination of mares with frozen-thawed semen provided acceptable pregnancy rates when barren mares were <16 yr of age. These results are similar to those obtained by Vidament.\(^2\) Pregnancy rates for insemination of mares with frozen-thawed semen were numerically similar to those bred with fresh semen. Loomis and Squires’ reported similar fertility with cooled versus frozen-thawed semen. The variable response to hCG in older mares was not surprising, because it is generally accepted that older mares respond poorly to hCG.\(^2\) It seemed that the presence of uterine fluid after insemination was not detrimental to fertility as long as mares were treated with oxytocin and/or uterine lavage. Thus, if one suspects uterine fluid in response to being inseminated, then ultrasound exams should be made 6–12 h later, and the required treatments should be performed. Similar to previous studies,\(^2,4,8\) two inseminations per cycle increased fertility over only one insemination.

Pregnancy rates of barren mares inseminated with frozen semen are reasonable and similar to artificial insemination with fresh semen as long as mares are >16 yr of age. Treatment with oxytocin and/or uterine lavage seemed to improve fertility.

References