Error in pregnancy diagnosis by per-rectal palpation in beef cows
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Abstract
The objective of this study was to determine the error rate of pregnancy diagnosis while differentiating artificial insemination (AI) pregnancy from bull-bred pregnancy in a synchronized breeding program. Angus-cross beef cows (N=1941) were artificially inseminated using Ovsynch-CIDR or CO-Synch-CIDR synchronization protocols. Bulls were introduced two weeks after AI to breed cows that were not pregnant from AI. The pregnancy status was diagnosed and days in gestation of each cow were determined by per-rectal palpation 70 d after AI. Projected gestation length was determined based on days of gestation at pregnancy diagnosis and projected calving date. The gestation length (281.2 ± 4.71 d; Mean ± SD) for the study population was retrospectively calculated from breeding and calving dates. These projected and actual gestation lengths were matched to determine the error in diagnosing pregnancies resulting from AI or by natural service. The error in pregnancy diagnosis was classified as error due to over-estimation (diagnosed pregnant to AI but pregnant to bulls) or under-estimation (diagnosed pregnant to bulls but pregnant to AI) using mean ± 2 SD as cut-off. The error rate for pregnancy diagnosis was estimated as 7.0 % (3.4% due to under-estimation and 3.6% due to over-estimation) in differentiating AI pregnancy from bull-breeding pregnancy for the gestation length of 281 days. Similarly, the error rate for gestation lengths of 279 and 283 days were estimated as 5.8% and 7.1%, respectively. The error rate varied from 4% to 13% among clinicians. In conclusion, estimation of pregnancy rate from AI breeding programs should account for the error in pregnancy diagnosis accuracy.

Keywords: Beef cows, breeding program, per-rectal palpation, pregnancy diagnosis, error rate

Introduction
Pregnancy is a measure of success in breeding programs. In clinical practice various diagnostic techniques have been adopted for diagnosing pregnancy. The most commonly used technique for diagnosing pregnancy is per-rectal palpation of the uterus and its contents.1-3 Other methods in use are transrectal ultrasonography,4,5 and measurement of progesterone5-7 pregnancy specific protein5,8 and early conception factors9 in serum. In beef synchronization programs, resynchronization of non-pregnant cows during the breeding season is not commonly utilized by producers due to repeated handling and difficulties in managing the cows. The most common practice is introduction of bulls two weeks after fixed-time AI (FTAI) to impregnate cows that are not pregnant from FTAI.11 This practice maximizes number of cows pregnant at the end of the breeding season while minimizing management. In order to measure the success of AI breeding programs it is necessary to distinguish FTAI pregnancy from bull-breeding pregnancy. It is a challenge to differentiate FTAI pregnancy from bull-bred pregnancy accurately by per-rectal palpation of the gravid uterus and its contents when the gestational difference is minimal. The objective of this study was to determine the error rate in determining cows pregnant to AI from cows pregnant to bulls by per-rectal palpation in a beef synchronization program.

Materials and methods
Cattle and synchronization protocols
Crossbred Angus beef cows (N=1941) were artificially inseminated using Ovsynch-CIDR or CO-Synch-CIDR synchronization programs. Briefly, cows were treated with 100 µg gonadotropin-releasing hormone (GnRH; Cystorelin®, Merial, Athens, GA) and a controlled internal drug release device (CIDR; Eazi-Breed™ CIDR®, Pfizer Animal Health, New York, NY) on Day 0, 25 mg dinoprost tromethamine (PGF; Lutalyse®, Pfizer Animal Health, New York, NY) and CIDRs were removed on Day 7 and either 100 µg GnRH 48 h after PGF on Day 9 (Ovsynch-CIDR), and FTAI 16
h after GnRH on Day 10 or 100 μg GnRH on Day 10 (CO-Synch-CIDR) at the time of FTAI. Angus bulls were penned with cows two weeks after FTAI (1:40 bull:cow ratio) to breed cows that were not pregnant for 45-50 days.

Pregnancy diagnosis
Pregnancy status was diagnosed for each cow by per-rectal palpation 70 d after FTAI. Once pregnancy was confirmed, the per-rectal palpation criteria used to differentiate FTAI pregnancy from bull-bred pregnancy (70 and < 55 d pregnancy, respectively) were: the size of the gravid horn (7.0 to 10.0 cm vs. 5.0 to 6.5 cm), presence or absence of fluid in the non-gravid horn, size of the amniotic vesicle (5.5 to 6.5 cm [extra-large hen egg size] vs. 3.5 to 4.5 cm [small hen egg size]). All criteria were used to confirm the gestational age. Six clinicians performed pregnancy diagnosis and their experience varied from one to 25 years.

Data management and analysis
Only cows inseminated with the semen from Angus sires were used in the analysis. The mean ± SD gestation length was 281.2 ± 4.71 d when cows that calved between 260 and 300 d after FTAI were considered. In order to prevent gestation length overlap between FTAI and natural service pregnancies, the pregnancy error study population was restricted. The mean ± SD time between FTAI and parturition in cows that conceived to bull breeding was 302 ± 4.71 d, only cows with gestation lengths of ≥ 271 d and ≤ 291 d were included in the analysis. Projected calving dates for a gestation length of 281 d were calculated based on estimated days of gestation at pregnancy diagnosis. These dates were compared to actual calf birthdates to determine error in pregnancy diagnosis. Error was classified as error due to over-estimation (diagnosed pregnant to AI but pregnant to bulls) or under-estimation (diagnosed pregnant to bulls but pregnant to AI) using the mean ± 2 SD as the cut-off. Similarly the error rate for gestation lengths of 279 and 283 d were also estimated. Influence of parity was examined using Chi-square analysis.

Data were analyzed with a statistical software program (SAS Version 9.1 for Windows, SAS Institute, Cary, NC). Descriptive analysis was performed to estimate mean gestation length. Arcsine transformation was applied to perform the analysis. The non-parametric one-way procedure of the SAS System was used to perform the Kruskal-Wallis test to compare the difference among clinicians for total error rate and error rate due to over-estimation and under-estimation. Influence of parity (2, 3, 4, and >4) was examined using Chi-square analysis.

Results
The error rate, based on mean ± SD, in differentiating FTAI pregnancy from bull-bred pregnancy is given in Table 1. The error rate for pregnancy diagnosis was estimated to be 7.0% (3.4% due to under-estimation and 3.6% due to over-estimation) in differentiating FTAI pregnancy from bull-bred pregnancy for the gestation length of 281 days. Similarly the error rate for gestation lengths of 279 and 283 days were estimated to be 5.8% (1.4% due to under-estimation and 4.4% due to over-estimation) and 7.1% (3.7% due to under-estimation and 3.4% due to over-estimation), respectively.

The error rate varied from 4% to 13% among clinicians. The year of graduation, number of animals examined and total error rate for clinicians are shown in Table 2. Frequency distribution histogram for gestation length based on actual calving date is shown in the Figure. There was no influence of parity on the error of pregnancy diagnosis.

<p>| Table 1. Error rate* in differentiating AI pregnancy from bull pregnancy (N=1941) |
|----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Gestation length (d)</th>
<th>Number under-estimated pregnancies</th>
<th>Error rate due to under-estimation</th>
<th>Number over-estimated pregnancies</th>
<th>Error rate due to over-estimation</th>
<th>Total number of errors</th>
<th>Total error rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>279</td>
<td>28</td>
<td>0.0144</td>
<td>85</td>
<td>0.0438</td>
<td>113</td>
<td>0.0582</td>
</tr>
<tr>
<td>281</td>
<td>66</td>
<td>0.0340</td>
<td>70</td>
<td>0.0361</td>
<td>136</td>
<td>0.0701</td>
</tr>
<tr>
<td>283</td>
<td>72</td>
<td>0.0371</td>
<td>65</td>
<td>0.0335</td>
<td>137</td>
<td>0.0706</td>
</tr>
</tbody>
</table>

* Mean gestation length ± 2 SD
Table 2. The year of graduation, number of animals examined and total error rate for clinicians who participated in the study

<table>
<thead>
<tr>
<th>Clinician</th>
<th>Year of Graduation</th>
<th>Number of animals</th>
<th>Total error rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1979</td>
<td>679</td>
<td>0.040 0.048 0.046</td>
</tr>
<tr>
<td>2</td>
<td>1993</td>
<td>369</td>
<td>0.043 0.055 0.060</td>
</tr>
<tr>
<td>3</td>
<td>1991</td>
<td>330</td>
<td>0.068 0.072 0.071</td>
</tr>
<tr>
<td>4</td>
<td>1984</td>
<td>233</td>
<td>0.076 0.077 0.080</td>
</tr>
<tr>
<td>5</td>
<td>2004</td>
<td>194</td>
<td>0.087 0.130 0.130</td>
</tr>
<tr>
<td>6</td>
<td>2004</td>
<td>136</td>
<td>0.095 0.120 0.120</td>
</tr>
</tbody>
</table>

Discussion

In this study, errors in assessing gestation length by per-rectal palpation to determine whether the cow was pregnant to FTAI or natural service were estimated. The error rate for pregnancy diagnosis was estimated to be 7.0 % for gestation lengths of 281 d. The mean (± SD) gestation length of 281.0 d (± 4.2) in the present study is consistent with that previously reported. In this study, the error due to over-estimation was similar to the error due to under-estimation (3.6% and 3.4%, respectively).

If all cows were successfully synchronized the difference in gestation length would be approximately 21 days between cows that conceived to FTAI and cows that conceived to the first service by bulls. However some cows may not have been synchronized and it is possible that the difference between an FTAI pregnancy and a bull-bred pregnancy may be as short as 14 days (bulls introduced 14 days after FTAI). Experience of the clinician performing the examination may account for some of the errors in estimating gestation length. In the present study the error rate decreased with increased years of experience. Other possible factors might be rumen fill, position of the uterus, ability of the examiner to retract the uterus and its contents, variation in the size of the gravid horn.
and size of the amniotic vesicle. Wisnicky and Cassida described per-rectal palpation of the amniotic vesicle as an aid in determining pregnancy status in cattle. Other investigators used crown-rump length and head and trunk diameters to determine gestational age using transrectal ultrasonography. These characteristics cannot be accurately employed in per-rectal palpation because vigorous and extended manipulation of uterus and fetus are required. In addition, these features are difficult to determine during 55 to 70 days of pregnancy due to the rigidity of amniotic vesicle.

The first report of pregnancy diagnosis in cattle by per-rectal palpation dated from the early 1800's. Currently per-rectal palpation is the most common method used for pregnancy diagnosis in cattle. In a beef cattle operation early detection of non-pregnant cows is the main benefit of pregnancy diagnosis. It allows producers to make critical management decisions several months prior to calving, including marketing decisions based on pregnancy status. It also allows determination of the success of AI programs. Veterinary schools across the United States and in other countries continue to train their students in the art of per-rectal palpation for diagnosis of pregnancy in dairy cattle. Because of its widespread use and the number of bovine practitioners trained to perform the procedure, per-rectal palpation will likely remain a mainstay for pregnancy diagnosis in cattle. Application of ultrasonography can be beneficial in determining gestation length. Even though ultrasonographic measurement of crown-rump length and head and trunk diameters is correlated with gestational age, measuring these parameters may be time-consuming. Features such as fetal and placentome size determined by ultrasonography may be useful to differentiate between gestational ages of 55 and 70 days. Estimations of error rate by transrectal ultrasonography and cost-benefit analysis are needed to determine the advantage of ultrasonography over per-rectal palpation for differentiating between a pregnancy due to AI or to natural service at a subsequent estrus.

The error in pregnancy diagnosis found in this study shows that it may influence the estimation of AI and synchronization program success. In conclusion, estimation of pregnancy rate in a beef AI program should account for the error in pregnancy diagnosis.

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References