The Effect of Artificial Insemination Timing on the Sex Ratio of Offspring and Fertility in Dairy Cows

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Received: 15.12.2005

Abstract: The aim of this study was to investigate the effect of the interval between artificial insemination and the onset of estrus on the sex ratio of offspring and fertility in Holstein cows. Standing to be mounted behavior was considered the beginning of estrus, and artificial inseinations were performed 6 h (Group 1), 9 h (Group 2), 12 h (Group 3), and 15 h (Group 4) after the onset of estrus in cows. Overall 119 (n: 85), 107 (n: 72), 120 (n: 80) and 119 (n: 83) artificial inseinations were performed in Groups 1, 2, 3 and 4, respectively. Pregnancy rates were 67.1%, 70.8%, 72.5% and 79.5% in Groups 1, 2, 3 and 4, respectively (P = 0.33). The highest female ratio of offspring and the pregnancy loss ratios were obtained in Group 2 but this difference was not significant (P = 0.54, P = 0.64). The highest twinning ratio was obtained in Group 4. The number of artificial inseinations per pregnancy was within the economical limits in all groups. In conclusion, the results of this study indicate that the interval between the onset of estrus and artificial insemination time does not alter the sex ratio of offspring in dairy cows with normal fertility parameters.

Key Words: Cow, artificial insemination time, sex ratio, fertility parameters

Introduction

The manipulation of the sex ratio of offspring in animal production systems is quite important for genetic improvement and economical development. Modified flow cytometry is a proven and reliable method for selecting the gender of offspring. However, it is still the most expensive method for commercial applications (1). In non-human mammals, primary and secondary sex ratios of newborns are thought to be affected by factors such as litter size, mother’s age, mother’s parity, mother’s parity in the previous breeding season, mother’s fecundity, maternal nutrition, mother’s milk yield, maternal stress, habitat quality, population demography, maternal dominance, paternal breeding success, date of birth, season of birth, time of insemination, type of service and inbreeding. Many studies have been carried out on these factors. The effect of the timing of artificial insemination on the sex ratio of offspring in cattle has been analyzed

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in the literature for approximately a century (2). In the last decade, many conflicting results have been reported on the effect of artificial insemination timing on the sex ratio of offspring in cows (3,4). Therefore, the present study investigated the effect of the time of artificial insemination on the sex ratio of offspring, pregnancy rate, pregnancy loss, twinning ratio, and the number of artificial inseminations per pregnancy in cows.

### Materials and Methods

#### Herd characteristics

This study was carried out on healthy Holstein cows (parity of the animals varied between 1 and 6) between 45 and 120 days postpartum in a dairy farm over 2 years in Sivas. The rolling herd average was 7200 kg per lactation. The cows were milked 3 times a day and received the same mixed ration consisting of corn silage, dry hay and concentrate.

#### Estrus detection and artificial inseminations

Estrus was observed by 2 experienced herders 3 times daily (at 08:00, 16:00 and 24:00) and each observation was approximately 30 min. The onset of estrus was determined when the cows stood to be mounted. Artificial inseminations were carried out by 2 veterinarian technicians with standard methodology for cattle. Semen was deposited in the body of the uterus. The cows were inseminated with frozen-thawed semen 6 h (Group 1), 9 h (Group 2), 12 h (Group 3) and 15 h (Group 4) after the detected onset of estrus. Overall, 119 (n: 85), 107 (n: 72), 120 (n: 80) and 119 (n: 83) artificial inseminations were performed in Groups 1, 2, 3 and 4, respectively. The cows were examined using a Honda Ultrasound machine with a 5 MHz transducer for pregnancy between 28 and 35 days after the artificial inseminations. In cases where the ultrasonographer was unsure of the diagnosis, another ultrasound examination was performed 1 week later. If a cow identified as pregnant showed estrus behavior, it was not inseminated and pregnancy was re-confirmed at the subsequent ultrasound examination. Failure to re-confirm pregnancy at a later ultrasound examination was identified as embryonic loss. The sex of the newborn calves was obtained from the veterinarian records of the farm.

#### Statistical analysis

Data were analyzed by using the Statistical Package for the Social Sciences (SPSS 11.5). Kruskal-Wallis and chi-squared tests were used to compare pregnancy rate, the sex ratio of offspring, pregnancy loss and the number of artificial inseminations per pregnancy. Statistical significance was taken as P less than 0.05.

### Results

The effect of artificial insemination timing on the sex ratio of offspring, pregnancy rates, pregnancy loss and artificial insemination per pregnancy is shown in the Table. The average sex ratio (female/male) of calves born was 48.2%/51.8% in all groups. The sex ratios (female/male) were 48.2%/51.8%, 56.3%/43.8%, 56.3%/43.8%.

<table>
<thead>
<tr>
<th>Groups (Artificial insemination time)</th>
<th>Group 1 (6 h)</th>
<th>Group 2 (9 h)</th>
<th>Group 3 (12 h)</th>
<th>Group 4 (15 h)</th>
<th>Test values</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy rate (%)</td>
<td>67.1</td>
<td>70.8</td>
<td>72.5</td>
<td>79.5</td>
<td>$X^2$: 3.41</td>
<td>0.33</td>
</tr>
<tr>
<td>Sex ratio (%) (female/male)</td>
<td>48.2/51.8</td>
<td>56.3/43.8</td>
<td>41.8/58.2</td>
<td>47.6/52.4</td>
<td>$X^2$: 2.15</td>
<td>0.54</td>
</tr>
<tr>
<td>Pregnancy loss (%)</td>
<td>3.4</td>
<td>7.3</td>
<td>4.9</td>
<td>6</td>
<td>$X^2$: 1.67</td>
<td>0.64</td>
</tr>
<tr>
<td>Twinning ratio (%)</td>
<td>1.81</td>
<td>2.12</td>
<td>0</td>
<td>4.5</td>
<td>*$X^2$: -</td>
<td>-</td>
</tr>
<tr>
<td>Artificial insemination number per pregnancy</td>
<td>1.40</td>
<td>1.47</td>
<td>1.46</td>
<td>1.43</td>
<td>**$KRX^2$: 0.49</td>
<td>0.92</td>
</tr>
</tbody>
</table>

* No statistical analysis could be performed to compare twinning ratios because the twinning ratio obtained in Group 3 was 0%  
** Kruskal-Wallis $X^2$ test
41.8%/58.2% and 47.6%/52.4% in Groups 1, 2, 3 and 4, respectively. Numerically, the highest female ratio was obtained in Group 2 and the highest male ratio was detected in Group 3, but these differences were not significant (P = 0.54). Pregnancy loss (P = 0.64) and artificial insemination per pregnancy (P = 0.92) and twinning ratio were similar among the groups.

**Discussion**

The control of the sex ratio of newborns is important for breeders to improve the yield of production. The effects of many factors (litter size, mother’s parity, mother’s milk yield, season of birth, timing of insemination, type of service and inbreeding) on the sex ratio of offspring have been investigated in dairy cattle. The influence of the timing of mating or insemination on the sex ratio of offspring has been the focus of many studies. However, scientists have reported many conflicting results about the effect of the timing of artificial insemination on the sex ratio of offspring in dairy cows (1-6).

Martinez et al. (3) suggested that the percentage of females in the offspring could be increased by the inseminations performed in the first 18 h after the onset of estrus. In contrast, the results obtained from the present study indicate that artificial inseminations performed at different times in the first half of the estrus period did not alter the sex ratio of offspring in dairy cows. The disparity between the results of these studies could be due to the differences in the detection of estrus, average milk yield inequality, the use of different artificial insemination protocols and the sire and quality of semen used for inseminations. Nonetheless, the estrus-insemination time interval was not as distinct as ours.

Pursley et al. (7) investigated the effect of the time of insemination on the sex ratio of calves by using the protocol of ovulation induction (Day 0 first GnRH, Day 7 Pgf2α, Day 9 second GnRH injection) and found that the female ratios were significantly higher in inseminations performed 0 and 32 h after the second GnRH injection. Furthermore, they reported that the sex ratios of newborns did not vary at 8, 16 and 24 h. The time of ovulation is between 24 and 32 h after the second injection of GnRH, which is similar to the time from the onset of estrus until ovulation. If the time of the injection of the second GnRH is considered the beginning of estrus in ovulation synchronization protocols, the results obtained from inseminations performed at 8, 16 and 24 h in this study could be thought of as compatible with our results.

Wehner et al. (8) investigated the efficiency of a device (OVATEC) improved for estrus detection in cows but researched the effect of breeding time on the sex ratio of offspring. They reported that the inseminations performed approximately 10 or 20 h before ovulation altered the sex ratio in cows. Nevertheless, they reported no influence of artificial inseminations performed with visual estrus observation and natural service on the gender ratios of offspring in cows. The sex ratio results in our study are compatible with the results obtained from the natural service and artificial inseminations of this study. The differences between the 2 studies may be due to the use of synchronization, the individual ovulation time differences and the type of semen used for inseminations in the cows.

In agreement with our results, Rorie et al. (1) reported that the insemination of beef cattle 10 or 20 h before expected ovulation does not alter the gender ratio of the resultant calves. Similarly, Foote (9) found no effect of the time of insemination on the sex ratio in heifers and cows. In addition, another study (10) carried out in pigs (as diverse species) has reported results similar to those of the present study.

Maatje et al. (11) reported that they obtained the highest conception rate between 6 and 17 h after the onset of estrus and stated that 11.8 h was the optimal time for insemination.

Similarly, Dransfield et al. (12) found the highest conception rate between 8 and 12 h after the onset of estrus. The highest conception rate was obtained at 15 h after the onset of estrus in the present study. Although 15 h is within the optimal interval, the pregnancy rate differences between these results could be due to the estrus detection methods and milk production differences. On the other hand, the pregnancy rates obtained from a previous study (13) investigating the non-return rates of artificial inseminations conducted daily and a.m.-p.m. in Holstein cows were lower than those of the present study. The higher pregnancy rate obtained from this study could be due to the number of inseminations and insemination time differences.
The mean artificial insemination per pregnancy detected in the present study is within the economical limits advised for healthy herds (14).

No statistical analysis could be performed to compare twinning ratios, because no twinning occurred in Group 3. The average twinning and pregnancy loss ratios in the present study were lower than those found in a previous study (15). This could be due to herd condition differences and rolling herd averages.

In conclusion, the interval between the time of artificial insemination and the onset of estrus (detected by visual observation) does not influence the gender ratio of offspring in Holstein cows.

Acknowledgments

The authors thank Mr Öztürk for his assistance with the statistical analysis, and Mr Koyuncu for his on-farm assistance.

References


