Effect of Embryo Transfer following Artificial Insemination (ETFAI) on Reproductive Performance in Dairy Cows in South-Western Japan

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ABSTRACT. In order to improve the reproductive performance during the summer period, embryo transfer (ET; Japanese black embryo) following artificial insemination (AI; Holstein semen: ETFAI) was conducted in dairy cows in south-western Japan (n=56). The conception rate was improved in cows with ETFAI compared with conventional AI, which served as the control (n=195; 30.4% vs. 13.8%, P<0.01). However, higher fetal loss was observed in ETFAI compared with the controls (38.1% vs. 7.4%, P<0.05). Four cases of twin pregnancy resulted in 2 singletons and a set of twins. There was no difference in the plasma progesterone level on d0 or d7 (d 0=AI), but rather lower rectal temperature was observed on d7 or d8 (38.7°C vs. 39.4°C, P<0.05) in pregnant cows compared with those that were open. ETFAI could improve reproductive performance in dairy cows during the summer period in south-western Japan.

KEY WORDS: dairy cow, ET following AI, fetal loss, rectal temperature, summer period.

Kumamoto Prefecture is ranked 4th in the Japanese dairy population and is located in the south-western Japan, one of the hottest areas in the country. In this area, the number of hot days when the diurnal temperature exceeds 35°C was more than 20 in 2004 and was 23 in 2006 (Japan Meteorological Agency) and reproductive performance in dairy cows was deteriorated, i.e., lower the conception rate, increase the number of days open or the number of artificial inseminations (AI) for conception. The calving interval in Kumamoto Prefecture is longer than that in Hokkaido (445 and 426 days, respectively; Livestock Improvement Association of Japan, LIAJ, 2008). Milk yield is also affected by heat stress, which is aggravated in high producing dairy cows [8, 10]. In general, oocytes [2] and embryos on d1 [4] to d2 [7] after breeding are sensitive to heat stress (d0=AI). But embryos on d7 are rather resistant to heat stress [14], and therefore embryo transfer (ET) might be considered in dairy industry [1, 12]. Takahashi et al. reported that a higher pregnancy rate was obtained with ET after AI in repeat-breeder Holstein cattle in the northern part of Japan, Hokkaido [13]. In the present study, we adopted ET following AI (ETFAI) in order to improve reproductive performance in dairy cows during the summer period in south-western Japan.

Profiles of the dairy farms: A total of 251 Holsteins kept at 13 commercial farms in Kumamoto Prefecture were used as the subjects in this study. Reproductive profiles of the 13 farms included 2.1 ± 1.3 AIs (mean ± SD) for conception, a calving interval of 422.3 ± 20.0 days, 159.2 ± 86.1 days in milk (DIM) and a BCS of 2.8 ± 0.4 on a 0.25 scale [5]. At these 13 dairies, the average number of lactating cows was 50 and the milk yield was 8,000–10,000 kg per lactation per cow. For cattle housing, three dairies had free stall barns and ten had tie stall barns. For feeding, separated feeding systems or total mixed rations (TMR) based on the CPM-Dairy system (developed by Cornell University, Penn State University and William H. Miner Agricultural Research Institute) were according to the guidelines of the NRC. Reproductive management: AI was performed in August and September of 2007. Among the 251 cows, 56 cows were classified into the ETFAI group, and their ovaries were palpated for the location of the corpus luteum 7 days after AI. Embryos were transferred into the uterine horn contralateral to the ovary of the corpus luteum. The remaining 195 cows were subjected to conventional AI as controls. This meant that one out of every five cows was selected at random for the ETFAI group. The DIMs of the ETFAI and AI group were 158.5 ± 79.2 days and 159.4 ± 88.6 days, respectively. Farm staff visually examined the cows every AM and PM in the cattle shed or milking parlor for sign of estrus. When estrus was observed, AI was performed by a veterinarian following the AM-PM method. Commercial semen straws of Holsteins were used, whereas in vitro fertilized (IVF) frozen embryos from Japanese black cows with excellent or good quality were transferred after freeze-thawing. Present study utilized in vitro fertilized frozen embryos (LIAJ). The AI or ET techniques and quality of semen or embryos were not varied among the trials. Rectal palpation and blood sampling were performed on d0 and d7. The rectal temperatures of the individual cows were measured (Ani-
mal Clinical Thermometer, Fujihira, Tokyo, Japan) in accordance with the ambient temperature and humidity (Pocket Weather Tracker, Kestrel 4000, Nielsen-Kellerman, Boothwyn, PA, U.S.A.) on d0, 1, 2, 3, 7, 8, 9 and 10. Pregnancy was determined on d38–d59 by rectal palpation and was reexamined by transrectal ultrasonography after d60. If an embryo/fetus was observed in the horn ipsilateral to the corpus luteum, we considered that the embryo might have originated from AI, whereas if the embryo/fetus was observed in the horn contralateral to the corpus luteum, we considered that the embryo might have originated from ETFAI. The calving conditions were carefully observed to determine the origin of new born calves, that is, whether they were Holstein calves from AI or Japanese black calves from ETFAI. Blood progesterone (P4) concentrations were measured by ELISA (KMK, Kawasaki Mitaka, Kanagawa, Japan). For statistical analysis, the Fisher’s exact test and Student’s t-test were used for comparison of the conception rate (CR), blood P4 level and body temperature using a commercial statistical software program for personal computers (Excel Statistics 2006 for Windows, SSRI, Tokyo, 2006).

Out of 56 cows, 17 cows conceived and calved 13 fetuses including a set of twins in the ETFAI group; this was significantly higher than for conventional AI, which resulted in 27 of the 195 cows conceiving (30.4% vs. 13.8%, P<0.01, Table 1). The conception rate (CR) showed no difference between free stall barns and tie stall barns (4/14 and 13/42 for the ETFAI group and 7/79 and 20/116 for the AI group, respectively). The blood P4 level showed no difference between the ETFAI and AI groups (13/56, 23.2% vs. 25/195, 12.8%). This high miscarriage rate (18.2% in AI and 60.0% in ETFAI) suggested that multiple pregnancies are inclined to result in more abortions [9]; in the present study, four twin cases resulted in only one set of twins being delivered. Poor quality embryos or embryos previously exposed heat stress are also risk factor for embryo/fetal loss [3].

Demetrio et al. [3] reported that embryonic loss was observed in 10.8% of embryos generated from AI and 21.5% generated from ET. More trials or further research are needed to solve this problem. Among the 17 pregnant cows in the AI/ETFAI group, 4 cows had twins; however, 3 of the 4 sets of twins were miscarried, and only 1 cow calved twins (Table 1). It is well known that multiple conceptions are associated with higher probability of miscarriage [9].

The blood P4 level showed no difference between the pregnant and open cows on d0 or d7 (0.74 ± 0.41 ng/ml vs. 0.90 ± 0.43 ng/ml on d0, p=0.357; 3.65 ± 2.09 ng/ml vs. 3.21 ± 2.27 ng/ml on d7, p=0.511; Fig.1). This result showed that the blood P4 levels on d0 or d7 were not directly related to conception during the summer period in dairy cows.

During the experimental period, the average temperature was 31.11 ± 2.24°C and the average humidity was 64.08 ± 10.11%. The temperature-humidity index (THI) was 81.84 ± 2.53. If THI exceeds a level of 72, it tends to have an adverse effect on the CR [11]; therefore, the present dairy

<table>
<thead>
<tr>
<th>Group (No. of cows)</th>
<th>Location of Embryo</th>
<th>No. of Embryos</th>
<th>No. of Calves</th>
<th>No. of Embryos/Fetuses loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Singleton</td>
<td>Twins (cows)</td>
<td>Singleton</td>
</tr>
<tr>
<td>ETFAI (56)</td>
<td>Ipsilateral (AI)</td>
<td>7</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Contralateral (ETFAI)</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>13</td>
<td>8(4)</td>
<td>9</td>
</tr>
<tr>
<td>AI (195)</td>
<td>Ipsilateral (AI)</td>
<td>27</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Contralateral (AI)</td>
<td>0</td>
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</table>

ETFAI: Embryo Transfer Following Artificial Insemination.

The number of cows pregnant at pregnancy diagnosis in the AI/ETFAI group was 17; 13 singletons and 4 set of twins (eight embryos in total) were detected.

Out of the 4 sets of twins in the AI/ETFAI group, 1 set of twins and 2 singletons were calved, however 1 set of twins was aborted during pregnancy.

A total of eight embryos/fetuses were lost during pregnancy, 2 from the ipsilateral horn (1 singleton and 1 set of twins) and 6 from the contralateral horn (3 singletons and 3 sets of twins) in the AI/ETFAI group.

Statistical differences were found in the pregnancy rate and embryo/fetus loss between the ETFAI and AI groups (17/56 [30.4%] vs. 27/195 [13.8%], P<0.01, and 8/21 [38.1%] vs. 2/27 [7.4%], P<0.05, respectively).
cows were under severe heat stress. On d7 or d8, the pregnant cows had significantly lower rectal temperatures than the open cows (38.86 ± 0.22°C vs. 39.35 ± 0.65°C for d7, p=0.004; 38.76 ± 0.31°C vs. 39.13 ± 0.52°C for d8, p=0.013; Fig. 1). These results suggest that rectal temperature may affect establishment of pregnancy around d7 or d8, which is in agreement with the results of a study by Demetrio et al. [3], which showed that a higher body temperature on d7 had a negative effect on conception or embryonic viability. Takahashi et al. [13] obtained higher pregnancy rates with ET following AI than in a stand-alone ET group in repeat-breeder Holsteins. The present study indicates that ET following AI could improve the conception rate in dairy cows even during the summer period in south-western Japan. However, ET following AI may possess other problems, such as higher embryonic death or abortion.

The heat stress on dairy cows in south-western Japan is increasing; however, there is currently no effective method to decrease the ambient temperature in barns or cool the body temperature. There are some controversial aspects of ETFAI, such as twins or embryo/fetal loss. However, if a fresh, high quality embryo could be used instead of a frozen embryo [1], ETFAI would become an innovative technology for improving reproductive performance in dairy cows during the summer period in south-western Japan.

REFERENCES


