



# Effects of parity and season on pregnancy rates after the transfer of embryos to repeat-breeder Japanese Black beef cattle

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**Abstract.** Repeat-breeder (RB) cows are a major source of economic waste due to their decreased fertility. Embryo transfer (ET) is an alternative tool to improve the fertility of RB cows. The aims of the present study were to evaluate the effects of recipient parity and the season on pregnancy rates following ET in RB Japanese Black beef cattle. Embryos were transferred nonsurgically to recipients, consisting of 155 heifers (<2 years old) and 172 cows (<8 years old), which were defined as RB cattle. Of the recipients that were presented for ET, 57 recipients received a fresh embryo and 270 recipients received a frozen embryo. There were no differences in the pregnancy rates between cattle that received fresh embryos or frozen embryos. The rates of recipients with pregnancy, abortion, stillbirth, and normal calving were similar between heifers and cows. In cows, the pregnancy rates were lower ( $P < 0.05$ ) in summer (June to August) than in spring (March to May) and winter (December to February). In heifers, however, there were no differences in the pregnancy rates among the seasons. Our findings indicate that in RB Japanese Black beef cattle, the parity of the recipients does not have an effect on the pregnancy rates following the transfer of fresh and frozen embryos. However, heat stress may affect reproductive performance in RB Japanese Black cows.

## 1 Introduction

Repeat breeding is considered to be one of the most important reproductive disorders in cattle. Repeat-breeder (RB) cows are defined as cows with three or more consecutive artificial inseminations (AIs) without conception and no clinical signs of disease (Gustafsson and Emanuelson, 2002). Although several causes of repeat breeding have been described, e.g. oestrus detection errors, endocrine dysfunction, and infections (Kendall et al., 2009; Moss et al., 2002; Perez-Marin and Espana, 2007), the particular reason for repeat breeding often remains speculative. Hormonal treatment, such as gonadotropin-releasing hormone (GnRH), has been used to increase the rate of pregnancy for RB cows (Kharche and Srivastava, 2007), but success has been limited. It has been demonstrated that embryo transfer (ET) can increase the probability of pregnancy in dairy cows by mini-

mizing the negative effects of high milk production and heat stress on both the quality of the oocyte and early development of the embryo (Vasconcelos et al., 2006). Moreover, the ET technique has been introduced as an efficient tool to improve fertility for RB dairy cows (Dochi et al., 2008; Son et al., 2007). These studies indicate that ET may be an effective technique to achieve satisfactory conception rates in RB beef cattle. However, limited information concerning the effects of ET on the pregnancy rates of RB animals is currently available for beef cattle because ET studies have been conducted mainly with dairy cows.

The aims of the present study were to evaluate the efficacy of ET in RB Japanese Black beef cattle and to investigate the effects of recipient parity and the season on pregnancy rates following ET.

## 2 Materials and methods

### 2.1 Management of embryo recipients

The animals receiving embryos were Japanese Black beef cattle, consisting of 155 heifers (<2 years old) and 172 cows with one or more deliveries (<8 years old), which were defined as RB animals. The RB animals were defined as animals that failed to conceive after three or more AIs. All RB recipients were raised in the Cattle Breeding Development Institute and on farms (Kagoshima, Japan), housed in a stanchion barn within an adequately wide area, and fed according to Japanese beef cattle feeding standards for beef cattle. The RB heifers with AIs (mean frequency: 7; range: 3–16) and RB cows with AIs (mean frequency: 6; range: 3–14) were selected from the farmers' herd records during regular herd visits. Only cattle with no clinical signs of uterine disorders, i.e. endometritis, and no abnormalities of the ovaries, i.e. ovarian cysts, were enrolled.

All procedures were approved by the Animal Research Committee of the Yamaguchi University and Cattle Breeding Development Institute of Kagoshima Prefecture. The owners of RB females had given permission for ET.

### 2.2 Embryo collection

Embryo collection was performed according to a method described by Isobe et al. (2013). All embryos were recovered from Japanese Black donors (2–15 years old), which were bred at the Cattle Breeding Development Institute or farms (Kagoshima, Japan). Superstimulation was induced by intramuscular administration of 18 AU follicle stimulating hormone (FSH; Kyoritsu Seiyaku Corp., Tokyo, Japan) that was administered in a series of decreasing doses over a 3-day period, beginning from day 10–14 of the oestrous cycle. Each donor was given two doses ( $375 \mu\text{g dose}^{-1}$ ) of synthetic derivative prostaglandin  $F_{2\alpha}$  (Cloprostenol C; Fujita Pharmaceutical, Tokyo, Japan) along with the fifth and the sixth FSH treatment. Donors were inseminated with frozen-thawed semen from Japanese Black bulls at 12 and 24 h after the detection of oestrus. Embryos were recovered nonsurgically using a balloon catheter 7 days after the first insemination. After collection, the embryos were classified according to the criteria defined by the International Embryo Transfer Society (Robertson and Nelson, 1998). The quality grade was assessed as good (grade 1), fair (grade 2), poor (grade 3), or degenerated (grade 4). Only good- and fair-quality embryos at the morula and blastocyst stage were used for freezing and ET.

### 2.3 Freezing and embryo transfer

Embryo freezing and transfer were performed by the procedures used by Isobe et al. (2013). The freezing medium consisted of 5% (*v/v*) ethylene glycol (EG; Wako Pure Chemical Industries Ltd., Osaka, Japan) and 6% (*v/v*)

propylene glycol (PG; Nacalai, Kyoto, Japan) in Dulbecco's phosphate-buffered saline (PBS; Invitrogen Corp., Carlsbad, CA, USA) supplemented with  $1 \text{ mg mL}^{-1}$  D-glucose,  $36 \mu\text{g mL}^{-1}$  sodium pyruvate (Invitrogen Corp.), 0.1 M sucrose (Nacalai), 0.5% sericin (Wako Pure Chemical Industries Ltd.), and 20% fetal bovine serum (Thermo Fisher Scientific, Worcester, MA, USA). Embryos at the morula and blastocyst stage were transferred directly into the freezing medium, and each embryo was then loaded into a 0.25 mL plastic straw (Fujihira, Tokyo, Japan) and allowed to equilibrate for 15 min. The straws were then placed in an alcohol bath of programmable freezer (ET-1; Fujihira) and pre-cooled at  $-6.5^\circ\text{C}$  for 5 min. Subsequently, seeding was induced by touching the straws with forceps pre-cooled in liquid nitrogen ( $\text{LN}_2$ ). Five minutes later, the straws were cooled to  $-30^\circ\text{C}$  at the rate of  $-0.3^\circ\text{C min}^{-1}$ , and then the straws were plunged into  $\text{LN}_2$  and stored in  $\text{LN}_2$ . After thawing the straw in a  $34^\circ\text{C}$  water bath, the frozen-thawed embryo was transferred directly to the recipient.

Either a single fresh (57 embryos) or frozen-thawed embryo (270 embryos) was transferred nonsurgically into the uterine horn, ipsilateral to the ovary bearing the corpus luteum, on days 6–8 of the oestrous cycle without hormonal treatments. Three trained technicians performed all of the transfers. The assessment of pregnancy was performed on days 30–40 after ET by transrectal ultrasonography (5.0 MHz transducer, Honda HS-101V; Honda Electronics Co., Aichi, Japan).

### 2.4 Statistical analysis

The percentages of cows that experienced pregnancy, abortion, stillbirth, and normal calving were analysed using a chi-square analysis with Yates' correction. To investigate seasonal effects on the pregnancy rates after the transfer of embryos, the results were compared among spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). The mean minimal and maximal average air temperatures between 2009 and 2014 in the experimental location were  $12.5$  and  $20.8^\circ\text{C}$  in the spring season,  $24.0$  and  $28.5^\circ\text{C}$  in the summer season,  $15.9$  and  $26.1^\circ\text{C}$  in the autumn season, and  $9.8$  and  $10.6^\circ\text{C}$  in the winter season, respectively. Differences with a probability value (*P*) of 0.05 or less were considered significant.

## 3 Results

When the data of heifer and cow recipients that experienced pregnancy were combined to assess any effect of embryo freezing, there were no differences in the pregnancy rates between cattle receiving fresh embryos and frozen-thawed embryos (31.6%, 18/57 vs. 31.9%, 86/270).

The rates of recipients with pregnancy, abortion, stillbirth, and normal calving were similar between heifers and cows

**Table 1.** Effects of parity on the rates of pregnancy, abortion, stillbirth, and normal calving after the transfer of embryos to repeat-breeder Japanese Black beef cattle.

Parity	No. of transfers	No. (%) <sup>1</sup> of pregnant cows	No. (%) of cows		
			abortion <sup>2</sup>	stillbirth <sup>3</sup>	normal calving <sup>4</sup>
Heifer	155	51 (32.9)	4 (7.8)	0 (0)	47 (92.2)
Cow	172	53 (30.8)	3 (5.7)	1 (2.0)	49 (92.5)

<sup>1</sup> Number of pregnant cows/number of cows with embryos transferred; <sup>2</sup> number of cows that aborted/number of pregnant cows; <sup>3</sup> number of cows with stillbirth/(number of pregnant cows – number of cows that aborted); <sup>4</sup> number of cows with normal calving/number of pregnant cows.

**Table 2.** Effects of the season on pregnancy rates after the transfer of embryos to repeat-breeder Japanese Black beef cattle\*.

Parity	Pregnancy rates (no. pregnant cows/no. cows with embryos transferred)			
	Spring	Summer	Autumn	Winter
Heifer	44.7 (17/38)	32.1 (9/28)	27.0 (10/37)	28.8 (15/52)
Cow	42.6 (23/54) <sup>a</sup>	17.1 (12/70) <sup>b</sup>	34.6 (9/26) <sup>a,b</sup>	40.9 (9/22) <sup>a</sup>
Total	43.5 (40/92) <sup>a</sup>	21.4 (21/98) <sup>b</sup>	30.2 (19/63) <sup>a,b</sup>	32.4 (24/74) <sup>a,b</sup>

\* Embryos were transferred to recipients in spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). <sup>a,b</sup> Values with different superscripts in the same row are significantly different ( $P < 0.05$ ).

(Table 1). When evaluating the effects of season on the pregnancy rates of recipients, the pregnancy rates in cows were lower ( $P < 0.05$ ) in summer than in spring and winter (Table 2). However, there were no differences in the pregnancy rates of heifers by season. The total pregnancy rate in summer decreased compared to that in spring.

#### 4 Discussion

The maternal environment of most RB cattle has been suggested to be satisfactory for establishing and maintaining pregnancy following ET (Tanabe et al., 1985). Our data showed that approximately 30% of RB beef cattle can become pregnant by ET, even if they did not conceive following AI. These results are in agreement with previous studies that ET is an alternative tool for the treatment of repeat breeding (Dochi et al., 2008; Son et al., 2007). Moreover, we found that parity did not affect the rates of pregnancy, abortion, stillbirth, and normal calving. In general, heifers are more appropriate recipients because they are less likely to be under nutritional stress or to have a history of compromised uterine or systemic health in the post-partum period (Broadbent et al., 1991). However, the pregnancy rates following the transfer of fresh and frozen embryos have been reported to be not affected by the parity of recipients (Hasler, 2001). Our findings also indicate that, in RB beef cattle, heifers are not expected to be more likely than cows to become pregnant after ET.

Oocytes and embryos at the early stages are extremely sensitive to heat stress (Ealy et al., 1993). The effect of heat

stress has been suggested to be due in part to the intrauterine environment, in which there is a decrease in blood flow to the uterus and an increase in uterine temperature (Roman-Ponce et al., 1978). These changes according to heat stress may inhibit embryonic development and increase early embryonic loss (Rivera and Hansen, 2001). However, embryos at 3 days and older are thought to be more resistant to heat stress than oocytes and early stage embryos. Several studies observed that ET can be applied to dairy herds to improve fertility compared with AI, particularly during the hot season of the year (Ambrose et al., 1999; Baruselli et al., 2011; Chebel et al., 2008). Therefore, ET is a strategy that bypasses the negative effects of heat stress during the periods of follicular development and early embryonic development. In the present study, we observed that the seasonal effect on the pregnancy rates following ET was different between heifers and cows. In heifers, the season did not affect the pregnancy rates in the recipients. In contrast, a significant difference was observed in the pregnancy rates of cows by season, resulting in a decreased pregnancy rate of recipients in the hot season. The reason for these discrepancies in the seasonal effect on the heifers and cows is unclear. Sartori et al. (2002) reported that cows had greater increases in body temperature in response to increases in environmental temperature than heifers. It has been suggested that an increase in environmental temperature that causes pronounced heat stress may augment the detrimental effects of negative energy balance in cows (Rensis and Scaramuzzi, 2003). Impairment of early embryonic development has been shown to be associated with a negative energy balance and poor body condi-

tion (Kendrick et al., 1999). Although nutrient requirements vary throughout the year, even in beef cattle (Laurenz et al., 1991), the variation in nutrient requirements for cows seems to be more than for heifers. Therefore, it is likely that heat stress affects the reproductive performance of cows directly and indirectly; the indirect actions may be mediated through alterations in energy balance. However, to identify the seasonal effects on the RB recipients, the relationship between energy balance and parity in beef cattle must be compared in future studies.

In conclusion, the results of this study indicate that the parity of recipients does not affect pregnancy rates following the transfer of fresh and frozen embryos in RB beef cattle. The hot season affected the pregnancy rates of cows. However, we confirmed that ET is an alternate treatment for Japanese Black beef cattle that are repeat breeders.

**Author contributions.** All authors contributed to the work described in the paper and all take responsibility for it. T. Ono, T. Isobe, Y. Morita, L. T. K. Do, F. Tanihara, M. Taniguchi, and M. Takagi as co-authors made a significant contribution to the conception and design of experiments, as well as the analysis and interpretation of data. Moreover, T. Otoi as a corresponding author participated in drafting the article as well as reviewing/ revising it for contents.

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