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Influence of season and parity on embryo recovery and subsequent reproductive performances in early postpartum suckling Japanese Black cows

Rajani Pradhan, K Oshima*, Y Ochiai*, T Kojima*, N Yamamoto*, M Elshabrawy Ghanem; *** and N Nakagoshi**

Graduate School for International Development and Cooperation, Hiroshima University, Kagamiyama 1-5-1, Higashi-Hiroshima, Hiroshima 739-8529, Japan
**National Agricultural Research Center for Western Region, Yoshinaga 60, Kawai, Oda, Shimane 694-0013, Japan*
***Laboratory of Animal Genetics, Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8528, Japan*
****Department of Theriogenology, Faculty of Veterinary Medicine, Suez Canal University Ismailia, Egypt*
rajanipradhan2003@yahoo.com ; rajanipradhan20033@gmail.com

Abstract

The objective of this study was to evaluate the effects of parity and season on the embryo recovery and subsequent reproductive performances in early postpartum suckling Japanese Black cows. A total of 95 cows of different parities (parity 2-3: n = 58; 4-5: n = 27; > 5: n = 10) in spring (n = 25), summer (n = 18), autumn (n = 38) and winter (n = 14) were used. Body weight of cows were measured before and after calving. Forty or 41 days after calving, cows were synchronized with controlled internal drug release device (CIDR), inserted for 7 days. After the onset of estrus, cows were artificially inseminated (AI) twice and embryos were recovered non-surgically on 7-8 days after AI. Cows which produced more than and less than 5 transferable embryos were categorized as Rank A and Rank B, respectively.

A higher number of freezable embryos were retrieved in parity 4-5 ($P < 0.05$). Significantly higher number of embryos were retrieved in winter than in spring, summer and autumn ($P < 0.05$). Rank A cows had significantly higher body weight before and after parturition, total number of embryos and corpus luteum (CL) than Rank B cows ($P < 0.05$). In conclusion, parity and season influenced embryo recovery in early postpartum suckling Japanese Black cows. Cows having high body weight, more CL produced good quality of embryos.

Key words: embryo quality, early postpartum, Japanese Black cows, parity, season

Introduction

An efficient reproduction in cattle herds is of great economic importance. During the past 30 years, embryo production has been a promising tool to enhance distribution of valuable genetics in different species of animals (Merton et al 2003). Embryo recovery offers many advantages to commercial animal breeding and also offers the chance to increase the overall rate of progress in genetic improvement to increase the productivity of a particular female and to shorten the genetic improvement. Initially much attention was placed on superovulation, non-surgical embryo recovery from donor animals that are rapidly succeeded (Greve et al 2005). Zavy et al (1994) indicated that embryonic mortality in cattle is the main source of economic loss for livestock producers. The proportion of oocytes developing to the blastocyst stage and quality of these embryos is important for embryo transfer. Putney et al (1988) reported that pregnancy rates varied slightly between seasons and that embryos should be collected from donor cattle during the cooler season. Heat stress has been shown to be harmful to bovine oocytes and embryo (Al-Katanani et al 1999, Wolfenson et al 2000). Although cattle ovulate and breed throughout the year, marked seasonal

differences in fertility have been reported in tropical and subtropical environments (Badinga et al 1985). Eduvie (1985) reported that calving interval is prolonged due to climatic stresses, season and parity. The season of calving and parity of animal influence reproductive efficiency (Sharma and Singh 1985). Season and parity are important constraints for the number and quality of embryos produced. Despite extensive research, the number and quality of embryos, the effect of parity and season on them is lagged behind. The objectives of this study were to investigate the effects of season and parity on the number and quality of embryos and subsequent reproductive performances on superovulated suckling postpartum Japanese Black cows.

Materials and methods

Study area

The study was conducted in experimental farm of National Agricultural Research Centre for Western Region in Japan. Animals were handled according to the guide for care and use of laboratory animals of this centre.

The experimental area was situated about 40 m above the sea level at a latitude and longitude of 35^{o}C, 27'N and 132^{o}C and 57'E. Four seasons were determined from climatic measurements: spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). The mean highest temperature during spring, summer, autumn and winter were 17^{o}C, 35^{o}C, 22^{o}C and 6.5^{o}C, respectively.

Animals

A total of 95 cows of different parities (parity 2-3: n = 58; 4-5: n = 27; > 5: n = 10) in spring (n = 25), summer (n =18), autumn (n = 38) and winter (n = 14) were studied. Animals were housed in open shed with access to a concrete floor, unshaded yard with shaded mangers and equipped with self-locking stanchions. They were fed a ration containing straw, silage and concentrate mixtures (crude protein: 13.5%, total digestible nutrient: 67.5%) using the feeding regimen to meet the maintenance, growth and lactation requirements in accordance with the Japanese Feeding Standard for beef cattle (MAFF 2000). Feeding was *ad libitum* and animals were locked to a stanchion while feeding. Mineral blocks and fresh water were available continuously through out the experimental period.

Body weight was measured before and after parturition. Cows were estrus-synchronized with CIDR (controlled internal drug release device) intravaginally, at 40 or 41 days after calving (Day 0 = the day of CIDR insertion) and superovulated using FSH (Kawasakimitaka, Kawasaki, Japan, 20 IU prepared by using Antrin 40) from day 5 to day 7 after insertion of CIDR, subcutaneously.

Artificial insemination for superovulation

Estrus was detected throughout the day. All cows were artificially inseminated (AI) twice using frozen semen of Japanese Black bull, after the onset of estrus according to a.m. and p.m. rule.

Ultrasonography

The ovaries and uterus of each cow were examined at embryo recovery by transrectal ultrasonography (ultrasound scanner equipped with 7.5 MHz transducer, Japan) and the numbers of CL were counted.

Embryo recovery

Embryos were collected non-surgically using approximately 1000 ml Ringer`s lactate solution with 1% calf serum on day 7 or 8 after the days of onset of heat. A balloon catheter, connected to two-

way tubing was inserted into the uterine horn through the cervix. A flushing solution was used to wash both uterine horn lumens. The recovered fluid was filtered through an embryo filter (200 mesh or 75 µm; Fujihara, Japan). Embryos were searched under stereomicroscope with approximately 10x magnification and transferred to holding media and then they were identified, evaluated and graded according to the morphological criteria of quality and viability determined based on the International Embryo Transfer Society Manual (Wright 1998). The cows with more than 5 transferable embryos were categorized as Rank A and those with less than 5 transferable embryos were categorized as Rank B. After flushing, recovered ova and embryos were separated into normal embryos, degenerate embryos and infertile ova. The normal embryos were categorized into five stages: Stage 1: morula, Stage 2: compact morula, Stage 3: early blastocyst, Stage 4: blastocyst and Stage 5: expanding blastocyst.

The following four quality grades (1-4) were used: Grade 1 (excellent: an ideal embryo, spherical, symmetrical with cells of uniform size, color and texture); Grade 2 (Good: trivial imperfection such as few extruded blastomeres, irregular shape, few vesicles); Grade 3 (Fair: definite but no severe problems such as few extruded blastomeres, vesiculation, few degenerated cells (10-30% irregularities); Poor (severe problems, such as numerous extruded blastomeres, degenerated cells, cells of varying sizes, large numerous vesicles but a viable appearing embryo mass (30-50% irregularities).

Reproductive performances after flushing

Following embryo recovery, cows that exhibited estrus were artificially inseminated according to a.m. and p.m. rule. The first AI conception rate, interval from embryo recovery to first estrus and conception were calculated. Days open was measured as number of days from calving to conception.

Statistical analysis

The difference between two means was tested using Student`s *t*- test for the body weight and reproductive performances in cows that produced more than or less than 5 transferable embryos. Data were analyzed using SAS (2003). When a significant effect was obtained using ANOVA, the significance of the difference between means was determined using Duncan`s multiple range test for parity and seasonal influences on embryo quality. In each case, *P* value less than 0.05 was considered as significant.

Results

Effect of parity

The influence of parity on embryo quality and subsequent reproductive performances is shown in Table 1.

Table 1. Effects of parity on embryo quality and subsequent reproductive performances in early postpartum suckling Japanese Black cows

Parameter	2-3	4-5	>5
Number of animals	n= 58	n= 27	n= 10
Total recovered ova and embryos	10.6 ± 0.9	12.8 ± 2.4	13.7 ± 2.9
Number of transferable embryos	8.1 ± 0.7	9.9 ± 1.8	11.4 ± 3.3
Number of freezable embryos	5.2 ± 0.5[b]	8.5 ± 1.7[a]	6.6 ± 1.8[b]
CL nos.	16.9 ± 0.9	18.8 ± 2.4	20.4 ± 3.1
Days open	93.5 ± 6.3	92.9 ± 7.8	86.5 ± 15.0
Days from embryo recovery to first estrus	15.3 ± 4.8	13.8 ± 3.4	9.0 ± 2.3
Interval from embryo recovery to conception, days	32.0 ± 4.5	41.1 ± 6.4	35.5 ± 12.2

First AI conception rate, %	76.3	53.3	60.0

[a,b] Means with different superscript differ in the same row (P <0.05)

The number of freezable embryos were significantly higher in parity 4-5 although there were no significant differences in total number of recovered ova and embryos and transferable embryos. After embryo recovery, interval to first estrus was longer in parity 2-3 than others even though no significant differences were found. Interval from embryo recovery to conception was longer in parity 4-5 than other parities (P > 0.05). Although there was no significant difference, the conception rate within first AI was higher in parity 2-3.

Effect of season

Seasonal influence on embryo recovery and its quality are summarized in Table 2.

Table 2. Seasonal influence on total number and quality of embryos in early postpartum suckling Japanese Black cows

Parameters	Spring	Summer	Autumn	Winter
Number of animals	25	18	38	14
Total number of recovered ova and embryos	9.6 ± 1.2^{b}	8.4 ± 1.2^{b}	12.0 ± 1.4^{b}	16.8 ± 4.2^{a}
Number of transferable embryos	8.1 ± 1.0	6.6 ± 1.2	6.0 ± 1.0	8.1 ± 2.5
Number of freezable embryos	7.3 ± 1.0	4.9 ± 1.3	4.6 ± 0.9	7.1 ± 2.2
CL nos.	18.5 ± 1.3	15.2 ± 1.3	17.3 ± 1.5	20.0 ± 4.0

[a,b] Means in the same row with different superscript differ significantly (P <0.05)

The total number of ova and embryos recovered were significantly higher in winter than in spring, summer and autumn (P < 0.05). The highest numbers of transferable and freezable embryo were obtained during winter and spring than in summer and autumn. However, the differences were not statistically significant. The numbers of CL detected by ultrasonography were higher in winter than in other seasons but statistical differences were not found.

Reproductive performances after flushing in Rank A and Rank B cows

Relationship of cows ranked A or B with their body weights and reproductive performances after flushing are presented in Table 3.

Table 3. Body weight (BW) and reproductive performances after embryo recovery in Rank A and B cows

Parameter	Rank A	Rank B
Number of animals	56	39
BW before parturition, kg	521.8 ± 6.7^{a}	511.3 ± 7.8^{b}
BW after parturition, kg	489.9 ± 6.4^{a}	477.5 ± 7.7^{b}
CL nos.	19.9 ± 1.2^{a}	14.3 ± 1.3^{b}
Days open	92.7 ± 6.1	100.1 ± 6.6
Total number of ova and embryos	14.1 ± 1.0^{a}	7.0 ± 1.0^{b}
Days from embryo recovery to first estrus	10.4 ± 1.3	13.2 ± 2.2
Interval from embryo recovery to conception, days	36.0 ± 6.1	43.3 ± 6.6
First AI conception rate, %	68.6	69.6

[ab]Means with different superscript differ in the same row (P <0.05)
Rank A: cattle that produced more than 5 transferable embryos
Rank B: cattle that produced less than 5 transferable embryos

The body weight before and after parturition were significantly higher in Rank A than in Rank B cows (P < 0.05). The numbers of CL and total number of recovered ova and embryos were significantly higher in Rank A than in Rank B cattle (P < 0.05), respectively. There were no

statistically differences in days to first estrus and days to conception after embryo recovery. Moreover, days open and conception rate were not statistically differences between two groups.

Discussion

The total number of ova and embryos and transferable embryos were higher in > 5 parity whereas number of freezable embryos were significantly higher in 4-5 parity. Ax et al (2005) reported that percentage of heifers that produced transferable embryos did not differ significantly however, the success rate increased with increasing age. This agrees with the present study as number of recovered ova and embryos and transferable embryos were higher in more than four parities even though no significant differences have been observed. Fertilization occurred at a similar rate among all age groups but the developmental capacity of embryo may have been compromised in younger animals (Ax et al 2005). According to Walters et al (2002), oocyte quality increased with the increasing number of oocytes retrieved which supports our study as transferable embryo numbers were in increasing pattern from 2-3 to > 5 parity. However, there might be limitation in oocyte recovery at certain parity and also factors like body weight, nutrition, breed and genetic conditions might have influence on it. Hasler et al (1983) noted that number of ovulated eggs were declined in cows older than 10 years old. On the other hand, Walters et al (2002) demonstrated that in first parity cows, because of stress in early lactation, the quality of embryos were lowest. Short et al (1990) found that cows at 2 years of age tend to have a longer postpartum interval which supports our result as the interval from embryo recovery to first estrus was longer in 2-3 parity but there were no significant differences. Postpartum ovulation occur earlier in cows that had calved more than twice compared to cows that calved two or less times (Eduvie 1985). Goshu et al (2007) found that cows after first calving required longer calving interval than cows in third or above parity. In agreement with this, the result of present study showed that days open was longer in 2-3 parity than that in 4-5 and more than 5 parity cows. There were no significant differences in parities with total recovered ova and embryos, number of transferable embryos and reproductive performances after flushing. This might be due to small number of animals used in this experiment.

Our results showed that total number of ova and embryos were influenced by season at which embryos were recovered. The total number of ova and embryos were higher in winter than in other seasons. Rutledge et al (1999) emphasized that production of cattle blastocyst was reduced in mid to late summer and high yield of blastocyst during winter. Total number of ova and embryos were lower in summer than in spring and autumn but no significant differences were observed. Rehman et al (1994) reported that high ambient temperature and humidity have deleterious effects on oocyte capability for maturation and fertilization. The ability of zygotes to develop blastocyst was reduced during summer (Al-Katanani et al 2002). In the present study, there was no significant difference in total number of ova and embryos in summer and spring. This might be due to small number of animals used in this experiment. Ryan et al (1993) found that there were no significant differences between hot and cool season in embryo/oocyte recovery rates on 6 or 7 days after estrus.

In current study, body weights before and after parturition were higher in Rank A than in Rank B cows. Fulkerson et al (2001) found positive relationship between body weight and the pregnancy rate. Body weight changes were confounded with embryo growth (Selk et al 1988). Cows that maintained body weight before and after parturition reserved ample energy which in turn exhibited estrus sooner than cows that had poor energy reserved due to low in considerable body weight (Selk et al 1988). Kendrick et al (1999) found that insufficient energy intake in early lactation can delay cyclicity and compromise follicle competence and embryo quality. Santos et al (2007) reported that inability to consume sufficient nutrients delayed resumption of ovulation, reduced the number of follicles and compromised oocyte quality. In our result, Rank A had high body weight that might have been due to storage of high energy which thereby increased the frequency of LH pulses, follicle maturation, and ovulation and enhanced in the production of more good quality embryos. Diameter of preovulatory follicles has been negatively correlated to weight loss in *Bos indicus* which in turn reduced in blastocyst development (Rhodes et al 1995). The low number of good quality of transferable embryos in Rank B cows with low body weights may be related to the low

level of energy taken by cattle. In this study, all cows were maintained under MAFF (2000) recommendations. Ryan et al (1994) noted that animals with low BCS have a lower IGF-I concentration than animals with intermediate scores. IGF- I increases the sensitivity of granulose cells to gonadotrophin and help maturation of oocytes and development of early embryos (Mariana et al 1991). The lower number of transferable embryos with low body weight might be due to lower IGF-I concentration. Further studies concerning details on nutrition related to embryo quality are necessary.

Embryologists utilize palpation of CL at the time of recovery to predict the number of embryos. The observed high numbers of CL in Rank A cows might lead to more secretion of progesterone and that have a positive effect on numbers and quality of embryos. According to Thatcher et al (2003), CL formation increase progesterone and reduce estradiol production with a consequent positive effect on embryo survival.

In conclusion, cows with more than four parities produced more numbers of total, transferable and freezable embryos. Although there are seasonal influences on total number of embryos, good quality of embryos can be collected year round in early postpartum suckling Japanese Black cows. Furthermore, cows which produced more than five transferable embryos showed high body weight before and after parturition.

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