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Clinical Study Report on Milk Production in the Offspring of a Somatic Cell Cloned Holstein Cow

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Abstract. This study examined two female offspring of a somatic cell cloned Holstein cow that had reproduction problems and milk production performance issues. The two offspring heifers, which showed healthy appearances and normal reproductive characteristics, calved on two separate occasions. The mean milk yields of the heifers in the first lactation period were 9,037 kg and 7,228 kg. The relative mean milk yields of these cows were 111.2% and 88.9%, respectively, when compared with that of the control group. No particular clinical abnormalities were revealed in milk yields and milk composition rate [e.g., fat, protein and solids-not-fat (SNF)], and reproductive characteristics of the offspring of the somatic cell cloned Holstein cow suggested that the cloned offspring had normal milk production.

Key words: Holstein cow, Milk production, Offspring of Somatic cell clone

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Cloning involves transferring the cell's nucleus into an enucleated mature oocyte, fusing the two components together using electric stimulation and transferring the resultant reconstructed embryo into the uterus of recipient cows, where it develops until birth. Cloned offspring were first produced in sheep in 1986 using nuclear transplantation of embryo cells [1], and this was subsequently achieved in cattle [2]. A sheep successfully calved a somatic cell cloned animal in Britain in 1996 [3] and this was later achieved in a cow in Japan [4].

Although the reproductive characteristics [5–7] and milk production performance [7] in somatic cell cloned Holstein cows have been examined, there are no reports on the reproduction and milk production characteristics of the offspring of a somatic cell cloned Holstein cow. It is known that abnormal phenotypes of somatic cell cloned mice are not inherited by the clone's descendants [8, 9]. In addition, the offspring of a somatic cell cloned bull did do not inherit the same abnormalities as the cloned bull [10]. These findings suggest that all abnormalities in the somatic cell cloned cow are eliminated in the following generations through reprogramming during gametogenesis [9, 11]. Because the offspring of a somatic cell cloned cow showed healthy appearances and had normal productivity, their milk production performance was studied to determine the possibility of industrial use.

In this study, reproduction and milk production performance of two offspring (CO1 and CO2) of Cody 2 were examined to verify the normality of the cloned offspring. The reproductive characteristics, the primipara age of the month or calving intervals (days) of the offspring and their somatic cell cloned Holstein cow were examined (Table 1). Furthermore, each offspring cow conceived twice, and no particular abnormal reproduction characteristics, such as in primipara age or calving interval, were found in the offspring of Cody 2.

The actual milk yields during the 305 days of each lactation period and the milk composition rates [e.g., fat, protein and solids-not-fat (SNF)] for CO1 and CO2 are listed in Table 1. The lactation curve and transitions of monthly milk compositions in the first lactation period for Cody 2, CO1 and CO2 are shown in Fig. 1. No difference was observed in the monthly milk yield between the offspring cows. The mean milk yields of CO1 and CO2 were 9,037 kg and 7,228 kg, respectively, with the mean milk yield of the control group corresponding to 8,128 kg (Table 1). Because the mean milk yield of the offspring cows were 111.2% and 88.9% of the control group means, they were considered to possess similar milk production performance as the control cows. Furthermore, because the offspring cows did not possess identical genes to the somatic cell cloned cow or the same reproductive background, a generalized comparison could not be made with the donor cow. However, for reference, the offspring cows produced 82.0% and 65.6% of the mean milk yield produced by the donor cow. Although the milk production performance of Cody 2 was considerably reduced, the offspring of Cody 2 had normal milk production performance. A variance of 7.1–16.7 kg in the transition of monthly milk yield between the offspring cows and Cody 2 during their first lactation period was verified (Fig. 1), representing a substantial difference in milk production characteristics. Accordingly, the abnormalities in milk production performance seen in the somatic cell clone, Cody 2, were not observed in the offspring of Cody 2.

The inconsistencies found in milk fat ratios (Fig. 1) could be largely attributed to seasonal factors and roughage quality. Proteins

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Table 1. Milk yield and milk compositions [fat, protein and solids-not-fat (SNF)] for each lactation period and calving intervals of the offspring (CO1, CO2) and their somatic cell cloned cow (Cody 2)

	Lactation period	Milk yield* (a, kg)	a/b** (%)	Fat (%)	Protein (%)	SNF (%)	Calving interval (days)
Somatic cell cloned cow	First	3,897	47.9	3.9	3.3	8.7	(26)***
Cody 2	Second	6,312	77.7	4.6	3.1	8.4	690
	Third	8,086	99.5	4.4	3.0	8.2	903
Mean		**6,098**	**75.0**	**4.3**	**3.1**	**8.4**	**797**
Offspring of Cody 2							
CO1	First	7,994	98.4	3.7	3.3	9.1	(25)***
	Second	10,079	124.0	5.4	3.0	8.5	494
Mean		**9,037**	**111.2**	**4.6**	**3.2**	**8.8**	**-**
CO2	First	6,837	84.1	3.9	3.3	9.1	(25)***
	Second	7,618	93.7	5.1	3.0	8.6	342
Mean		**7,228**	**88.9**	**4.5**	**3.2**	**8.9**	**-**
Mean (CO1+CO2)		**8,132**	**100.0**	**4.5**	**3.2**	**8.8**	**418**

The milk yields during the 305 days of each lactation period. ** Percentage in relation to the mean milk yield (b: 8,128 kg, n=563) of the control cow group. *** The primipara age of the month.

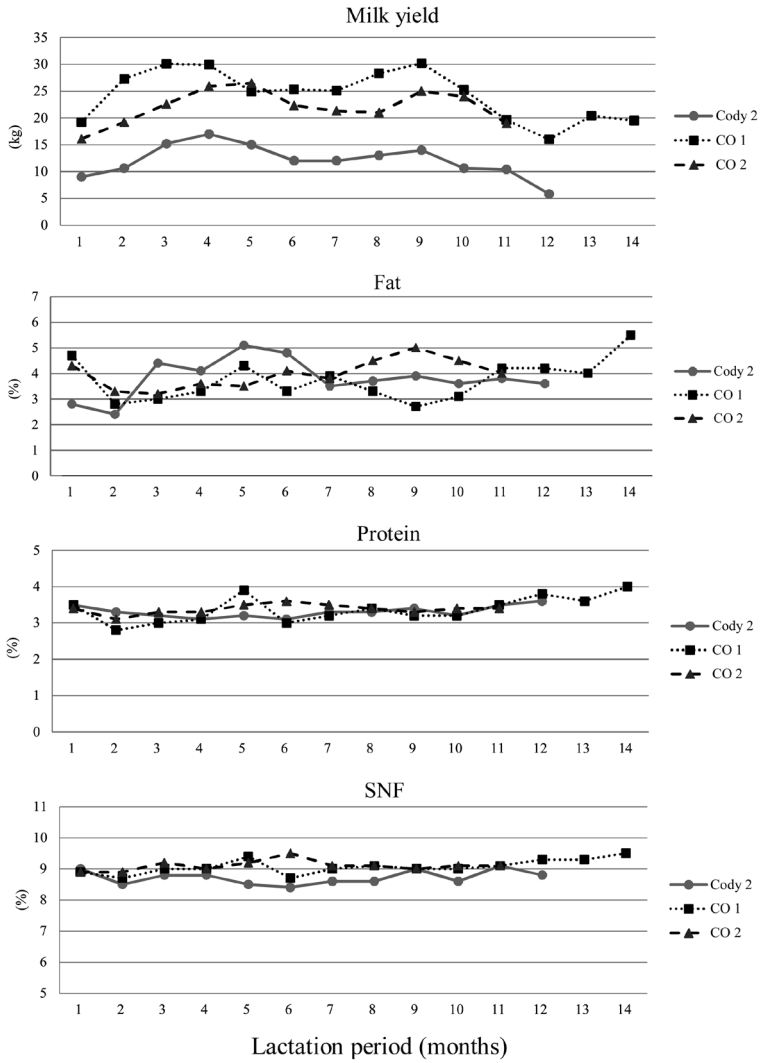

Fig. 1. Lactation curve and transitions of monthly milk compositions [fat, protein and solids-not-fat (SNF)] for Cody 2, CO1 and CO2 during the first lactation period.

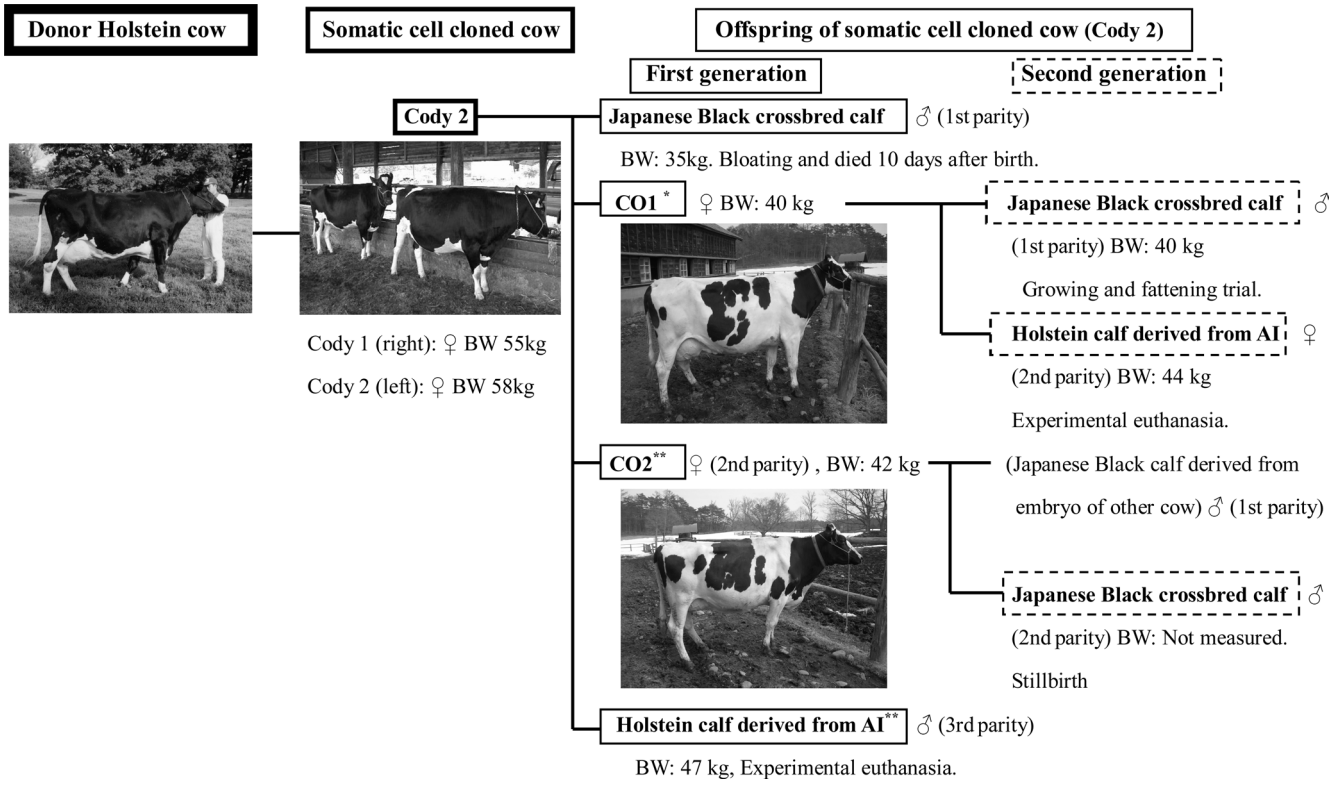

Fig. 2. Birth weights (BW) and other after-birth data of the somatic cell cloned Holstein cow (Cody 2) and the offspring are shown in the pedigree charts. * Holstein cow derived from multiple ovulations for Cody 2 and embryo transfer. ** Holstein cow and calf derived from artificial insemination (AI) for Cody 2.

and SNF ratios were stable, with little deviations. Typical milk compositions for domestic cows are 85%–88% water, 3.0%–4.0% crude proteins, 4.6%–5.2% lactose and 3.0%–5.0% fats [12]. Mean milk compositions in both the offspring and Cody 2 were within the normal range, as outlined by Walstra [12], suggesting that there were no abnormalities in the milk composition levels in either the offspring or their somatic cell cloned cow. According to Tian [13], the milk and meat compositions of somatic cell cloned cattle did not differ from those of regular cattle.

In this study, abnormalities in reproduction or milk production performances were not observed in the offspring of the somatic cell cloned cow, and the collected data constituted additional pieces of evidence that the offspring of the cloned cow were healthy. DNA microarray analysis has been performed to determine the precise genome-wide gene expression profiles of somatic cloned mice [14]. The results demonstrated unexpectedly large epigenetic diversity in neonatal cloned mice, despite their normal appearance and genetic identity. Therefore, gene expression may be aberrantly controlled in the somatic cell cloned cow with low reproductive performance and milk production. Gluckman reported that a part of the epigenetic changes in the mammalian fetus is transmitted to progeny [15]. However, the abnormal phenotype in somatic cell cloned mice was not transmitted to their offspring [8, 9]. Somatic cell cloned animal progeny can eliminate differences in the epigenetic changes by gametogenic reprogramming [11]. Therefore, the abnormalities of the parent somatic cell cloned Holstein cow did not persist in the two offspring, which would have happened by conventional breeding.

Somatic cell cloned cows do not constitute safety and quality issues related to meat and milk as food products. However, because the consumer's confidence in cloned food items is still uncertain, the distribution of meat or milk products produced through somatic cell cloning technology remains largely disregarded in Japan even today. Despite this, cloning technology allows for fast arrangement of highly productive cattle groups, and it can be greatly beneficial to highly increase the production efficiency of milk and meat. In this study, the offspring of a somatic cell cloned cow demonstrated healthy and normal milk production performance; therefore, these cloned offspring may be used for industrial food production. Using cloning technology, such problems as the global food crisis may potentially be solved, and species that are extinct can potentially be revived. Therefore, we hope that cloning technology will be utilized once again in Japan in the future.

Methods

In this study, somatic cell cloned embryos were successfully produced on the basis of a previous report [16]. Briefly, femoral fibroblasts of an 11-year-old Holstein dairy cow from Koiwai Farm were used as donor cells for somatic cell cloned embryos. These embryos were then transferred into recipient cows in the same farm

facility. Two cloned Holstein cows, Cody 1 and Cody 2, were derived from femoral fibroblasts of the donor Holstein cow with the somatic cell cloning technique. Birth weights (BW) and other after-birth data of the offspring of Cody 2 and of Cody 2 are shown in the pedigree charts (Fig. 2). Heifers CO1 and CO2 were the offspring of Cody 2. CO1 was born after multiple ovulation treatments on Cody 2 and embryo transfer into recipient cows, whereas CO2 was born through artificial insemination (AI) of Cody 2. In addition, the sires of CO1 and CO2 differed. Growth performance of the two somatic cell cloned heifers did not differ from that of other cattle born within the same month, with no specific apparent clinical abnormalities.

The second generation offspring of the somatic cell clone heifers calved successfully, with CO1 producing a male Japanese Black crossbred calf in the first parturition and then producing a female Holstein calf via AI in the second parturition. Both calves appeared normal and healthy at birth. CO2 delivered a stillbirth male Japanese Black crossbred calf in the second calving.

Reproduction and milk production data were collected from the donor cow (first–sixth lactation), Cody 2 (first–third lactation), CO1 and CO2 (first–second lactation). Milking cows from Koiwai Farm (563 cows with a mean parity of 2.6) were used as the control group. The primipara age of the month and calving intervals (days) were collected as reproduction data. The calving intervals (days) of the donor Holstein cow (first–sixth lactation period) and the control group were 523 days and 427 days, respectively. The reproduction performance was particularly reduced in the two somatic cell cloned Holstein heifers [17].

For milk productivity, data for the lactation curve and transitions of monthly milk compositions during the first lactation (Fig. 1) were collected on the basis of the dairy herd performance test every month. The milk yield (kg) over 305 days and milk composition rates (fat, protein and SNF) for each lactation period were measured (Table 1). In addition, the cows were milked twice per day. The mean milk yield and milk composition rates (fat, protein and SNF) of the donor Holstein cow from the first to sixth lactation periods were 11,015 kg and 3.5%, 3.2% and 8.7%, respectively, whereas they were 8,128 kg and 3.7%, 3.3% and 8.9%, respectively, for the control group.

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