Journal of Reproduction and Development, Vol. 57, No. 5, 2011

—Original Article—

Relationships of Survival Time, Productivity and Cause of Death with Telomere Lengths of Cows Produced by Somatic Cell Nuclear Transfer

Kazuyuki KONISHI[1)#], Miharu YONAI[1)##], Kanako KANEYAMA[1)], Satoshi ITO[1)###], Hideo MATSUDA[1)], Hajime YOSHIOKA[1)####], Takashi NAGAI[2)] and Kei IMAI[1)]

[1)]*National Live Stock Breeding Center, Fukushima 961-8511 and* [2)]*National Institute of Livestock and Grassland Science, Ibaraki 305-0901, Japan*
[#]*Present: National Live Stock Breeding Center Tottori Station, Tottori 689-2511, Japan*
[##]*Present: National Agricultural Research Center for Tohoku Region, Iwate 020-0124, Japan*
[###]*Present: National Live Stock Breeding Center Niikappu Station, Hokkaido 056-0141, Japan*
[####]*Present: National Live Stock Breeding Center Miyazaki Station, Miyazaki 886-0004, Japan*

Abstract. The reproductive ability, milk-producing capacity, survival time and relationships of these parameters with telomere length were investigated in 4 groups of cows produced by somatic cell nuclear transfer (SCNT). Each group was produced using the same donor cells (6 Holstein (1H), 3 Holstein (2H), 4 Jersey (1J) and 5 Japanese Black (1B) cows). As controls, 47 Holstein cows produced by artificial insemination were used. The SCNT cows were artificially inseminated, and multiple deliveries were performed after successive rounds of breeding and conception. No correlation was observed between the telomere length and survival time in the SCNT cows. Causes of death of SCNT cows included accidents, accident-associated infections, inappropriate management, acute mastitis and hypocalcemia. The lifetime productivity of SCNT cows was superior to those of the controls and cell donor cows. All SCNT beef cows with a relatively light burden of lactation remained alive and showed significantly prolonged survival time compared with the cows in the SCNT dairy breeds. These results suggest that the lifetime productivity of SCNT cows was favorable, and their survival time was more strongly influenced by environmental burdens, such as pregnancy, delivery, lactation and feeding management, than by the telomere length.
Key words: Clone cow, Productivity, Somatic cell nuclear transfer, Survival time, Telomere length

(**J. Reprod. Dev. 57:** 572–578, 2011)

Calves produced by somatic cell nuclear transfer (SCNT) tend to be lost at a high rate from the perinatal period until about 6 months after birth, as well as through mortality due to abortion [1, 2]. However, their growth thereafter is considered similar to normally bred cows [3-5]. The mortality has been assumed to be due to large offspring-associated abnormalities and malformation of the heart and lungs during the perinatal period [5-7]. The incidence of abnormalities of respiratory and motor organs has been reported to be high up to 150 days after birth [3, 4]. However, there have been limited reports on the survival time and cause of death in SCNT cattle without clinical abnormalities that grew to breeding age. The relationship between survival time and telomere length has been controversial [8-13]. We previously reported that there are no relationships between telomere length and development and reproductive and lactation characteristics among 2 groups of dairy breed SCNT cows [14]. There have been fewer reports on the relationships between telomere length and productivity. Moreover, there are no reports on the relationship between the actual survival time and productivity in SCNT cattle.

In this study, we surveyed the survival time and cause of death in 18 SCNT cows in 4 groups, including 10 cows of 2 groups (6 Holstein and 4 Jersey cows) observed in our previous report [14] and newly cloned Holsteins (3 cows with measurements of telomeres) and Japanese Black cattle (5 cows without measurements of telomeres). The relationships of telomere length with reproductive ability and milk-producing capacity are described.

Received: November 9, 2011
Accepted: April 27, 2011, 2011
Published online in J-STAGE: June 10, 2011

Correspondence: K Imai (e-mail: k0imai@nlbc.go.jp)

Materials and Methods

Test cows

The test cows included a total of 18 cows in 4 groups produced using donor cells provided by 4 cows of 3 breeds: 6 Holstein cows (1H group), 3 Holstein cows (2H group), 4 Jersey cows (1J group) and 5 Japanese Black cows (1B group). The SCNT cow groups and their sources of donor cells and individual cows are shown in Table 1. The 1H and 1J groups were investigated in our previous report [14]. For the control cows, 47 Holstein cows produced by artificial insemination and born between April 2001 and March 2002 were used.

Nuclear and embryo transfer

SCNT embryos were prepared by the method reported by Goto *et al*. [15]. In brief, cumulus-oocyte complexes (COCs) were aspirated from abattoir-derived ovaries and matured *in vitro* for 20 h in TCM-199 (Gibco BRL, Grand Island, NY, USA) supplemented with 5% calf serum (CS: Gibco BRL). Mature COCs were treated with 0.5% hyaluronidase (Sigma, St. Louis, MO, USA) in M2

Table 1. List of somatic cell nuclear transfer (SCNT) cows

SCNT group (Source of donor cell)	Animal number	Birth date	Measurement of telomere length	
			White blood cell telomere length (kb)	Age at sampling (months)
1H Holstein (Oviduct epithelial cell)	Donor	1986/10/6	15.2	137.6
	H1	1999/2/15	12.9	9.9
	H2	1999/2/23	13.4	9.6
	H3	1999/2/23	13.2	9.6
	H4	1999/5/5	13.7	7.2
	H5	1999/5/13	12.5	6.9
	H6	1999/11/22	14.2	0.6
		Total SCNT	13.3 ± 0.6	7.3 ± 3.6
1J Jersey (Oviduct epithelial cell)	Donor	1992/5/1	16.9	68.3
	J1	1998/12/8	15.9	8.1
	J2	1998/12/8	14.5	8.1
	J3	1999/4/13	14.9	3.9
	J4	1999/4/13	16.0	3.9
		Total SCNT	15.3 ± 0.7	6.0 ± 2.4
2H Holstein (Cumulus cell)	Donor	1993/7/30	No data	–
	H11	2001/4/13	19.3	3.2
	H12	2001/5/14	18.0	2.2
	H13	2001/5/16	18.6	2.1
		Total SCNT	18.6 ± 0.7	2.5 ± 0.6
1B Japanese Black (Cumulus cell)	Donor	1998/6/8	No data	–
	CB1	2001/9/11	No data	–
	CB2	2001/9/12	No data	–
	CB3	2001/9/13	No data	–
	CB4	2001/11/21	No data	–
	CB5	2001/12/6	No data	–

medium [16] for 5 min, and the cumulus cells were removed by gentle pipetting. The oocytes were transferred to PBS supplemented with 20% CS and 5 μg/ml of cytochalasin B (Sigma). The zona pellucida of each denuded metaphase II oocyte was cut near the polar body, and the cell was enucleated by pushing the nucleus and surrounding cytoplasm out of the cell with a glass needle. Enucleation was confirmed using the nuclear stain Hoechst 33342 (Sigma). Donor oviduct epithelial or cumulus cells were introduced into the perivitelline space of enucleated oocytes with a microinjection glass pipette, and then the cell-oocyte complexes were transferred to Zimmerman cell fusion medium [17]. Fusion of the cell-oocyte complexes was accomplished by applying one pulse of 25 V for 50 μsec. After 30 min of the fusion procedure, fused complexes were exposed to 5 μM calcium ionophore (A23187, Sigma) for 5 min and then incubated for 6 h in TCM-199 supplemented with 5% CS and 10 μg/ml cycloheximide (Sigma). After the activation treatment, the nuclear-transplanted oocytes were cultured for 8 days in CR1aa medium [18], supplemented with 5% CS to the blastocyst stage. The quality of the blastocysts was assessed, and those ranked as good to excellent were used for embryo transfer. All SCNT cows were confirmed to be derived from cows that provided donor cells (donor cows) by DNA testing.

Telomere length measurement

The telomere length was measured by using the method reported by Miyashita *et al*. [11]. DNA was extracted from white blood cells and labeled with telomere-specific oligonucleotides, and the length was determined employing chemiluminescence analysis. Measurements were not performed for the 1B group.

Feeding and reproduction management

Feeding and conventional breeding of the 1H, 1J and 2H groups were managed as previously reported [14]. All SCNT cows were fed in accordance with NRC standards during the experimental period. The 1B group was similarly managed until the suckling period, but the site of feeding was transferred to a free stall barn during the rearing period and managed according to the Japanese Feeding Standard for Beef Cattle (2000).

All groups were artificially inseminated with frozen semen after reaching the breeding age, and breeding was repeated. Artificial insemination was subjected to a voluntary 80-day waiting period after delivery for the dairy breeds. Breeding was repeated until the sixth and fourth parity for the1H and 2H groups, respectively, and until the third parity in the 1J and 1B groups, and breeding was stopped thereafter for convenience of management. Breeding of the control group was not limited. Breeding for primiparity was later than usual in the 1B group because the animals were used for a superovulation treatment test when they became nulliparous.

Table 2. Survival time, number of deliveries and survival days after the final delivery in somatic cell nuclear transfer (SCNT) cows

Group (breed)	Animal number	Outcome	Survival time (years)	Number of deliveries	Survival days after the final delivery
1H (Holstein)	Donor	Culled	11.5	2	–
	H1	Death	6.7	5	33
	H2	Death	8.0	6	25
	H3	Death	6.2	4	237
	H4	Death	7.7	6	161
	H5	Death	6.0	4	255
	H6	Death	4.4	2	353
		Total SCNT	6.5 ± 1.3	4.5 ± 1.5	
1J (Jersey)	Donor	Culled	5.7	3	–
	J1	Culled	5.0	3	295
	J2	Alive	11.8	3	2,762
	J3	Death	4.5	3	3
	J4	Death	5.3	3	312
		Total SCNT***	4.9 ± 0.4	3.0 ± 0.0	
2H (Holstein)	Donor	Slaughtered	11.4	2	–
	H11	Culled	8.8	4**	1,224
	H12	Death	3.2	1*	432
	H13	Death	5.8	4	17
		Total SCNT	5.9 ± 2.8	3.0 ± 1.7	
1B (Japanese Black)	Donor	Slaughtered	4.8	0	–
	CB1	Alive	9.1	3	1,604
	CB2	Alive	9.1	3	1,508
	CB3	Alive	9.1	3	1,497
	CB4	Alive	8.9	3	1,607
	CB5	Alive	8.8	3	1,521
		Total SCNT	9.0 ± 0.1	3.0 ± 0.0	

* Died after the second conception. ** Including 1 abortion. *** Excluding surviving cows.

Surveyed items

When the SCNT cows were involved in accidents or became morbid, symptomatic treatment was performed, but the animals were euthanized if the prognosis was deemed to be poor. The survival time was investigated in dead or euthanized cows. Seven of the 18 test cows were alive at the time of the survey (October 1, 2010). Autopsy findings were collected for all dead cows (natural death or euthanized). In addition, the number of deliveries and the time (days) of death after the final delivery were investigated. The conditions immediately before death were recorded. The milk yield was investigated for 305 days until the third parity in the 1H and 1J groups and for the first parity in the 2H group. For convenience of management, the 1H and 2H groups were dried off after a short-term milking period (100 or 150 days) or immediately after delivery. The survival time, milk yield (305 days) and number of deliveries were similarly surveyed for each donor cow. In the control group, animals with acute diseases and those involved in accidents were culled as well as those not fertilized within 1 year after a previous delivery and those with low-level milk-producing capacity. The survival time or timing of culling after birth was investigated as well as the milk yield for cows that could be milked for at least 305 days.

Statistical analysis

The correlation coefficient between the telomere length and survival time, reproductive ability and milk-producing capacity in the SCNT cows was determined, and the significance was tested using simple linear regression analysis. The difference of survival time between dairy breeds and beef breeds was analyzed by Welch's t-test. P values less than 0.05 were considered to indicate statistical significance.

Results

The telomere lengths measured in the 1H, 1J and 2H groups are presented in Table 1. In the production states of the SCNT cow groups (test cows), the final production rates of test cows from the recipient cows in the 1H, 2H, 1J and 1B groups were found to be 9.5, 7.7, 18.2 and 12.5%, respectively.

Survival time, reproductive states and telomere length

At the time of the survey, all animals of the 1H and 2H groups and 3 animals of the 1J group were dead. The survival time, number of deliveries and time (measured in days) of death after the final delivery of the individual SCNT and donor cows are presented in Table 2. The mean survival times in the 1H, 2H and 1J groups were

Table 3. Conditions before death and autopsy findings in the individual SCNT and donor cows

Group (breed)	Animal number	Conditions before death and cause of death	Autopsy findings
1H	Donor	The animal was culled based on the poor prognosis of acute mastitis.	
(Holstein)	H1	This cow died during the drying-off process after the fifth delivery. Acute mastitis developed 4 days after delivery and was cured by treatment. However, the animal suddenly died about 1 month after delivery.	Heart failure, abscess formation in the mammary region and hepatitis
	H2	This cow died during the drying-off period after the sixth delivery. Fever developed soon after initiation of the drying-off period. The cow was markedly emaciated and became unable to stand 3 days before death. Respiratory insufficiency may have been the direct cause of death.	Nephritis, hepatitis and a large volume of ascites
	H3	This cow died about 100 days after the short milking period (150 days) following the fourth delivery. The cow became unable to stand 3 weeks before death, and emaciation became aggravated despite the treatment, resulting in debilitation of the cow. Deviation of the abomasum, which may have been caused by falling, was also concomitantly present.	Costal and sternal fractures and 4 basketball-sized abscesses in the thoracic and abdominal cavities
	H4	This cow died as a result of an accident involving a scraper in a barn. The accident occurred about 5 months after the sixth delivery.	Heart failure
	H5	This cow died about 100 days after the short milking period (150 days) following the fourth delivery. The cow became unable to stand and died during fluid replacement. Before death, abrasion of the body surface, chronic mastitis and emaciation were observed.	Valvular heart disease, heart failure, hepatitis, nephritis and pulmonary emphysema
	H6	This animal died during the drying-off period after the third conception. It was involved in an accident resulting in one of its legs being caught in a fence. The cow became unable to stand and died on the same day during fluid replacement.	Heart failure, pulmonary emphysema and hepatitis
1J (Jersey)	Donor	This cow was culled because its value as a breeder had decreased.	
	J1	This cow became unable to stand due to an abnormality of the hind leg later during the lactation period after the third delivery. The animal was euthanized based on the poor prognosis.	Fractures of both iliac bones
	J2	This cow was alive at the time of submission of this paper.	
	J3	This cow became unable to stand due to hypocalcemia on the day after the third delivery and died 2 days later during calcium fluid replacement.	Heart failure
	J4	This cow died during the drying-off process after the milking period following the third delivery. The autopsy indicated acute mastitis as the cause of death.	Acute mastitis
2H (Holstein)	Donor	This cow was euthanized as a result of diagnosis of an accidental rupture of the femoral head ligament and surrounding muscle.	
	H11	This cow became unable to stand due to arthritis in the right hock.	Accumulation of serous fluid in the capsule of a joint and calcareous deposit in the pulley of talus
	H12	This cow died after acute mastitis developed immediately before the second delivery.	Sepsis
	H13	Acute mastitis occurred immediately before the fourth delivery. Mastitis was cured by treatment, and the cow gave birth, but fever developed due to retention of the placenta, and the cow became unable to stand and died.	Sepsis and a circulatory disorder
1B (Japanese Black)	Donor	This cow was euthanized because of fat necrosis.	Fat necrosis around the rectum
	CB1	This cow was alive at the time of submission of this paper.	
	CB2	This cow was alive at the time of submission of this paper.	
	CB3	This cow was alive at the time of submission of this paper.	
	CB4	This cow was alive at the time of submission of this paper.	
	CB5	This cow was alive at the time of submission of this paper and had fat necrosis around the rectum.	

Table 4. Death and culling of the somatic cell nuclear transfer (SCNT) and control groups (cows born in fiscal 2001)

	Outcome	Number of cows	Parity	Age at death (years)	Age at decision to cull (years)
SCNT (n = 18)	Death or Cull	12	3.8 ± 1.5	6.0 ± 1.6	–
	Alive*	6	3.0 ± 0.0	9.4 ± 1.2**	–
Control (n = 47)	Death or Cull	15	1.9 ± 0.8	4.5 ± 1.4	–
	Slaughtered (reproductive disorder and low ability)	32	1.8 ± 1.2	–	4.3 ± 1.5

* Alive but not used for breeding. ** Age at the time of the survey.

6.5 ± 1.3, 4.9 ± 0.4 and 5.9 ± 2.8 years, excluding the cow that was still alive. The reproductive states of each SCNT group were as follows. In the 1H group, the mean number of deliveries recorded was 4.5 ± 1.5 times, and the mean time of conception after the previous delivery was 134.0 ± 43.0 days (in a total of 21 cows), showing that pregnancy and delivery were relatively smoothly repeated. In the 1J group, there were 3 deliveries in total. The mean time of conception after delivery was 121.9 ± 35.2 days (for a total of 8 cows). This shows smooth repetition of conception and delivery, and the mean age at the third delivery was 4.3 ± 0.1 years. In the 2H group, the mean age of the 3 cows at the first delivery was 2.1 ± 0.1 years, and conception was achieved in all cows after the first delivery. However, one cow died immediately before the second delivery. Breeding was repeated until the fourth parity for the other 2 cows. Since abortion occurred and conception after the second delivery was slightly delayed for one of the cows, the mean time of conception after delivery was 171.5 ± 59.8 days (for a total of 7 cows). In the 1B group, there were no clinical abnormalities in any of the 5 cows at the time of survey at 9.0 ± 0.1 years of age. Breeding was repeated until the third parity for all 5 cows. The mean age at the third delivery was 4.7 ± 0.2 years, and the mean time of conception after delivery was 85.1 ± 36.8 days (for a total of 10 cows). This indicates favorable repetition of conception and delivery.

A correlation was not observed between the survival time and telomere length in the individual dairy breed SCNT cows (r= –0.14, n=13, P=0.659). In the survival time, there was a significant difference (P<0.01) between dairy cows (1H, 1J and 2H group) and beef cows (1B group), and a significantly difference (P<0.01) was found between the 1H and 1B groups. And, there was no correlation between the days for conception after primiparity and telomere length in the dairy breed SCNT cows (r=0.23, n=13, P=0.454).

Time of death, conditions before death and autopsy findings

The time of death, conditions before death and autopsy findings in the individual SCNT and donor cows are described in Tables 2 and 3.

1H group: All the SCNT cows (H1 to H6) of the 1H group were dead at the time of this study. Two cows died during the drying-off process immediately after the final delivery. Other cows died as a result of accidents, formation of large abscesses such as sequelae of accidental trauma, extreme emaciation and mistreatment. Heart failure was noted 4 of 6 cows in autopsies.

1J group: Three of 4 cows were dead at the time of this study. One cow died as result of hypocalcemia during the perinatal period, and other cows died or were culled as a result of acute mastitis during the dry-off process or accidents such as fractures of both iliac bones.

2H group: All the SCNT cows (H11 to H13) of the 2H group were dead or had been culled at the time of this study. Two cows of this group died as result of acute mastitis during the perinatal period, and the other cow died by accident with estrus behavior.

1B group: All the SCNT cows (B1to B5) were alive at the time of submission of this paper.

Milk yield

1H group: The mean milk yield after 3 deliveries by 6 different cows within a group of 17 cows was 10,622 ± 1,387 kg, and that of the donor cow for 2 milking periods was 11,205 kg.

1J group: The mean milk yield for 3 deliveries by a total of 11 cows was 6,894 ± 848 kg, and that of the donor cow for 3 milking periods was 6,254 ± 1,282 kg.

2H group: The milk yield for the first delivery was more than 10,000 kg in 2 of the 3 cows, but less than 4,000 kg in the remaining cow. This resulted in a mean milk yield of 8,791 ± 4,220 kg. The reason for the extremely low milk yield in the latter cow was unclear. The milk yield of the donor cow was 10,437 kg (only for the first delivery).

The mean milk yield in the control group milked for 305 days was 10,580 ± 1,428 kg (n=42, mean parity per cow: 1.5 ± 0.7). There was no correlation between the milk yields and telomere length in the Holstein breed SCNT cows (r= 0.23, n = 13, P=0.454), and no correlations were observed among the individual cows in the 1H group (r=0.75, P=0.09), 1J group (r= –0.89, P=0.11) and 2H group (r=0.87, P=0.33).

Follow-up of the control group and comparison with the SCNT cows

The conditions of the control group (47 cows) at the time of survey are summarized in Table 4. Fifteen cows (31.9%) died as a result of a specific disease, accident, euthanasia or emergency cull. The mean age and parity at the time of death in these 15 cows were 4.5 ± 1.4 years and 1.9 ± 0.8, respectively. All of the remaining 32 cows were culled. Of the 18 SCNT cows, 12 (66.7%) were dead at the time of the survey. In the remaining 6 cows, breeding was stopped after 3–4 deliveries for convenience of management, and their survival was monitored. The mean age and parity at the time of culling and survey are shown in Table 4.

Discussion

Regarding the cause of death of the SCNT cows, heart failure was frequently noted in autopsies of cows of the 1H group derived from the same donor cells. However, other causes including accidents, formation of large abscesses such as sequelae of accidental trauma, extreme emaciation and mistreatment also led to death. In the 1J group, the causes of death, in addition to accidents, were hypocalcemia and mastitis, which occurs at a high incidence in Jersey cows. The cause of death was acute mastitis in 2 cows in the 2H group. Failure of feeding management and treatment may have been indirect causes, and the acquired factors may have been the main causes. No clinical abnormalities occurred during the suckling or raising period in any of the 18 SCNT cows, and even the shortest survival time was 3.2 years. Since there are no previous reports on the survival time, cause of death and conditions before death in SCNT cows [19], it is difficult to draw conclusions regarding the survival of SCNT cows.

The results of this research showed that the survival time tended to be shortened in the dairy breeds of SCNT cows relative to the survival times of donor cows and that the mortality tended to be higher in the SCNT cows than in the control group, for which the following reasons were considered. In this survey, breeding was repeated until the fourth and sixth parity in the 1H and 2H groups, respectively. It is of interest that the survival time and mean parity of the cows of the dairy breed groups were as follows: 6.5 years and 4.5 in the 1H group, 4.9 years and 3.0 in the 1J group and 5.9 years and 3.0 in the 2H group. The survival time and mean parity of the cows of the control group were 4.5 years and 1.9. Thus, we discovered, for the first time, that the reproductive ability of the SCNT cows is markedly favorable. It should be noted that there are no previous reports in which the breeding state after the second delivery was investigated in SCNT cows. The mean milk yields were 10,622 ± 1,387, 6,894 ± 848 and 8,791 ± 4,220 kg in the 1H, 1J and 2H groups, respectively. Those of Holstein and Jersey cows in Japan in 2001–2005, the same period as this survey, were 7,400–7,900 and 5,700–6,000 kg, respectively. Since the mean of the control group was 10,580 kg, the milk yield of the SCNT cows is favorable relative to that of cows produced through normal reproduction. Based on the reproductive ability and milk yield, the lifetime productivity of the dairy breed SCNT cows may have been very high, which may subsequently have loaded a heavy burden on the body. In fact, six of eleven SCNT cows obviously died as a result of diseases related to delivery and lactation. It is difficult to discuss the survivability of the SCNT cows from these phenomena. However, it appears that the repeated pregnancies and lactation periods clearly reduced the survival time of the SCNT cows in the present study. It has also been reported that there was a negative correlation between the lifespan of cows and their milk yield [20, 21]. In fact, the survival times of the donor cows of the 1H and 2H groups that had 2 deliveries were long, and this may have been due to the possibility that the fewer deliveries may have caused less of a burden of pregnancy and lactation on the body. Furthermore, the members of the 1J group in which both the donor cow and SCNT cows had 3 deliveries showed similar survival times. Moreover, all SCNT beef cows with a relatively light burden of lactation remained alive (mean survival at the time of survey, 9.0 years) and showed a significantly prolonged survival time compare with the cows in the 1H group. Death of the dairy breed SCNT cows was induced by failure of management, suggesting that the severe burden of repeated pregnancy and lactation on the body and environmental factors, such as maintenance management, had a marked influence on the survival time. For example, in the 1J group derived from the same somatic cells, three cows died at similar ages (4.9 ± 0.4) as a result of accidents and acute mastitis; however, cow J2 has experienced no accidents, remains alive at 11.8 years of age and is the oldest daily SCNT cow in the world.

There have been few reports on the survival time of cows in general. This is not limited to SCNT cows. Ohgi and Hatta [22] surveyed the productive life-span of milking cows in Hokkaido and observed a low mean number of deliveries with annual culling and disease/injury/accident-associated damage rates as high as 25 and 81%, respectively, in groups with a milk yield of 9,000 kg or more relative to those with a low milk yield. The incidence of diseases of the lactating, reproductive and digestive organs was found to be markedly different between cows with high and low milk yields. Regarding the lifespan of SCNT cows, Wells *et al*. [19] reported that the annual mortality of SCNT cows until 4 years of age after weaning was 8% and that the main causes of mortality were abnormal skeletal muscle, tympanites, accidents, poisoning by feeds and parasitism. However, these causes of mortality were not specific to SCNT cows, and the data were insufficient to investigate lifespan, as reported by Chavatte-Palmer et al. [3]. In SCNT mice, the survival time was found to be shortened in some cases [23] but remained unchanged in others [24]. The immunological conditions during the milking period were investigated in the 6 SCNT cows in the 1H group, but no overall significant difference was noted relative to the control group [25]. In addition, a correlation was not observed between the survival time and telomere length in the individual dairy breed SCNT cows. These findings suggest that the causes of death of the SCNT cows were not specific or derived from the SCNT and that the telomere length does not markedly influence the survival time, reproductive ability or milk-producing capacity. This suggests that the survival time of SCNT cows is not influenced by genetic differences but rather environmental factors and the burdens of pregnancy, delivery and lactation.

Acknowledgments

We thank Dr N Miyashita (National Institute of Agrobiological Sciences) for performing the telomere length measurements, Dr T Kojima (Kagoshima University) for reviewing this manuscript and the staff of the productive hygiene section of our institute for treatment and autopsies of the cows included in this study.

References

1. **Farin PW, Piedrahita JA, Farin CE**. Errors in development of fetuses and placentas from *in vitro*-produced bovine embryos. *Theriogenology* 2006; 65: 178–191.
2. **Le Bourhis D, Chesne P, Nibart M, Marchal J, Humblot P, Renard JP, Heyman Y**. Nuclear transfer from sexed parent embryos in cattle: efficiency and birth of offspring. *J Reprod Fertil* 1998; 113: 343–348.
3. **Chavatte-Palmer P, Remy D, Cordonnier N, Richard C, Issenman H, Laigre P, Heyman Y, Mialot JP**. Health status of cloned cattle at different ages. *Cloning Stem Cells*

2004; 6: 94–100.

4. **Panarace M, Aguero JI, Garrote M, Jauregui G, Segovia A, Cane L, Gutierrez J, Marfil M, Rigali F, Pugliese M, Young S, Lagioia J, Garnil C, Forte Pontes JE, Ereno Junio JC, Mower S, Medina M**. How healthy are clones and their progeny: 5 years of field experience. *Theriogenology* 2007; 67: 142–151.
5. **Watanabe S, Nagai T**. Health status and productive performance of somatic cell cloned cattle and their offspring produced in Japan. *J Reprod Dev* 2008; 54: 6–17.
6. **Kato Y, Tani T, Sotomaru Y, Kurokawa K, Kato J, Doguchi H, Yasue H, Tsunoda Y**. Eight calves cloned from somatic cells of a single adult. *Science* 1998; 282: 2095–2098.
7. **Wells DN, Misica PM, Tervit HR**. Production of cloned calves following nuclear transfer with cultured adult mural granulosa cells. *Biol Reprod* 1999; 60: 996–1005
8. **Betts D, Bordignon V, Hill J, Winger Q, Westhusin M, Smith L, King W**. Reprogramming of telomerase activity and rebuilding of telomere length in cloned cattle. *Proc Natl Acad Sci USA* 2001; 98: 1077–1082.
9. **Jeon HY, Hyun SH, Lee GS, Kim HS, Kim S, Jeong YW, Kang SK, Lee BC, Han JY, Ahn C, Hwang WS**. The analysis of telomere length and telomerase activity in cloned pigs and cows. *Mol Reprod Dev* 2005; 71: 315–320.
10. **Lanza RP, Cibelli JB, Blackwell C, Cristofalo VJ, Francis MK, Baerlocher GM, Mak J, Schertzer M, Chavez EA, Sawyer N, Lansdorp PM, West MD**. Extension of cell lifespan and telomere length in animals cloned from senescent somatic cells. *Science* 2000; 288: 665–669.
11. **Miyashita N, Shiga K, Yonai M, Kaneyama K, Kobayashi S, Kojima T, Goto Y, Kishi M, Aso H, Suzuki T, Sakaguchi M, Nagai T**. Remarkable differences in telomere lengths among cloned cattle derived from different cell types. *Bio. Reprod* 2002; 66: 1649–1655.
12. **Ortegon H, Betts DH, Lin L, Coppola G, Perrault SD, Blondin P, King WA**. Genomic stability and physiological assessments of live offspring sired by a bull clone, Starbuck II. *Theriogenology* 2007; 67: 116–126.
13. **Schaetzlein S, Rudolph KL**. Telomere length regulation during cloning, embryogenesis and ageing. *Reprod Fertil Dev* 2005; 17: 85–96.
14. **Yonai M, Kaneyama K, Miyashita N, Kobayashi S, Goto Y, Bettpu T, Nagai T**. Growth, reproduction, and lactation in somatic cell cloned cows with short telomeres. *J Dairy Sci* 2005; 88: 4097–4110.
15. **Goto Y, Kaneyama K, Kobayashi S, Imai K, Shin-noh M, Tsujino T, Nakano T, Matsuda S, Nakane S, Kojima T**. Birth of cloned calves derived from cultured oviductal epithelial cells of a dairy cow. *Anim Sci J* 1999; 70: 243–245.
16. **Fulton BP, Whittingham DG**. Activation of mammalian oocytes by intracellular injection of calcium. *Nature* 1978; 273: 149–151.
17. **Zimmermann U, Vienken J**. Electric field-induced cell-to-cell fusion. *J Membr Biol* 1982; 67: 165–182.
18. **Rosenkrans CF Jr, Zeng GQ, MCNamara GT, Schoff PK, First NL**. Development of bovine embryos *in vitro* as affected by energy substrates. *Biol Reprod* 1993; 49: 459–462.
19. **Wells DN, Forsyth JT, McMillan V, Oback B**. The health of somatic cell cloned cattle and their offspring. *Cloning Stem Cells* 2004; 6: 101–110.
20. **Onyiro OM, Andrews LJ, Brotherstone S**. Genetic parameters for digital dermatitis and correlations with locomotion, production, fertility traits, and longevity in Holstein-Friesian dairy cows. *J Dairy Sci* 2008; 91: 4037–4046.
21. **Haworth GM, Tranter WP, Chuck JN, Cheng Z, Wathes DC**. Relationships between age at first calving and first lactation milk yield, and lifetime productivity and longevity in dairy cows. *Vet Rec* 2008; 162: 643–647.
22. **Ohgi T, Hatta T**. Effects of milk yield and feeding systems on death and illness incidence in 217 dairy herds in the Nemuro district of Hokkaido. *J Jpn Vet Med Assoc* 2001; 54: 667–672 (in Japanese).
23. **Ogonuki N, Inoue K, Yamamoto Y, Noguchi Y, Tanemura K, Suzuki O, Nakayama H, Doi K, Ohtomo Y, Satoh M, Nishida A, Ogura A**. Early death of mice cloned from somatic cells. *Nat Genet* 2002; 30: 253–254.
24. **Tamashiro KL, Wakayama T, Yamazaki Y, Akutsu H, Woods SC, Kondo S, Yanagimachi R, Sakai RR**. Phenotype of cloned mice: development, behavior, and physiology. *Exp Biol Med (Maywood)* 2003; 228: 1193–1200.
25. **Tanaka S, Miyazawa K, Watanabe K, Ohwada S, Aso H, Yonai M, Saito N, Yamaguchi T**. Comparison of T cell subsets between somatic cloned and normal cow. *Am J Reprod Immunol* 2006; 55: 28–35.