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Somatic Cell Cloned Cattle and Their Progeny Produced in Japan: A Report for Animal Health and Characteristics of Animal Products

体細胞クローン牛・後代牛の健全性ならびに生産物性状に関する国内調査報告書

独立行政法人
農業・食品産業技術総合研究機構
畜産草地研究所

NARO
National Institute of
Livestock and Grassland Science

Preface

Development of new technologies for animal reproduction is one of the important missions of National Institute Livestock and Grassland Science. Namely, we succeed in producing calves with innovative technologies including non-surgical embryo transfer (1964), cryo-preservation of embryos (1979), *in vitro* fertilization (1985), embryonic cell nuclear transfer (1990), embryo sexing using PCR (1992) and somatic cell nuclear transfer (1998). These innovative technologies have been transferred to local experimental stations, which were established by local governments, using visiting researcher program of our institute. Research activities of local experimental stations were also stimulated by bounty and technical training program of Japanese government. Local governments also promoted investments to their experimental stations concerning animal production, especially around 1990. Therefore, there were many local experimental stations which had excellent equipments and highly skilled researchers at the end of 1990s.

With such background, six local experimental stations succeeded in producing somatic cell cloned cattle in 1998, the year that the first somatic cell cloned cattle in the world was produced. At present, 33 local experimental stations produced somatic cell cloned cattle; there are 47 local governments in Japan. They produced 336 somatic cell cloned cattle; it is 57.1% of 588 clones produced in Japan (as of September 2010).

These somatic cell cloned cattle have been investigated by local experimental stations. The obtained findings were published in Japanese language. These reports were reviewed in No.9 issue of "Memories of National Institute of Livestock and Grassland Science"; however, this issue was also written in Japanese language. To provide information recorded in the No.9 issue into the world, its English version is published as a No.12 issue of the series.

The present issue might be instructive for coming risk assessments concerning somatic cell cloned animals and follow-ups of the past risk assessments.

March 2011

Mitsuto Matsumoto Ph. D
Director general
National Institute of Livestock and Grassland Science
National Agriculture and Food Research Organization

刊行にあたって

牛の新しい繁殖技術として，戦後間もない旧畜産試験場（現 農研機構 畜産草地研究所）において凍結精液に関する研究が開始された。その後，繁殖新技術の開発は，非外科的受精卵移植（1964），受精卵凍結（1979），体外受精（1985），受精卵クローン牛（1990），受精卵の性特異的な遺伝子の診断による雌雄産み分け（1992）などの成果をあげながら進展し，体細胞クローン牛（1998）の生産までに至った。その間に開発された新しい技術体系は，依頼研究員制度などを活用して速やかに都道府県に移転されていった。それと同時に，農林水産省の補助事業や技術研修制度，そして，畜産県を中心にした研究施設整備や研究員の海外留学など畜産研究への 1990 年前後の積極的な投資が相乗的に機能し，都道府県の繁殖技術水準が飛躍的に高められた。

これらを背景に，世界初の体細胞クローン牛が生産された 1998 年には，６県で体細胞クローン牛の生産に成功している。その後，これまで 47 都道府県のうち 33 都道県において体細胞クローン牛が生産されている（2010 年 9 月 30 日現在の農林水産省プレスリリースによる，以下同様。）。わが国は 588 頭の体細胞クローン牛生産実績を有する世界有数の「体細胞クローン牛生産大国」である。これを生産機関別に集計すると，都道府県：336 頭（57.1%），独立行政法人：191 頭（32.5%），民間企業：54 頭（9.2%），大学：7 頭（1.2%）に区分される。

生産された体細胞クローン牛の大部分は多方面にわたる調査や試験に用いられ，貴重なデータが蓄積された。これらは先に研究資料９号「体細胞クローン牛・後代牛の健全性ならびに生産物性状に関する国内調査報告書」として取り纏めた。しかし，資料自体，さらに引用あるいは参考とした原著の多くも日本語であることから，わが国における体細胞クローン研究の成果を世界に向けて広く情報発信することを目的として，この英語版研究資料を発行することとした。

体細胞クローン家畜とその後代に関するリスク評価を 2008 年に済ませている米国や欧州では，その評価結果をフォローアップする責務を果たすための新規データの収集と分析に余念がない。そのような場面で，あるいは，新規にリスク評価を行う場面で本研究資料は貴重な文献として活用されるものと考えている。

平成 23 年 3 月

独立行政法人　農業・食品産業技術総合研究機構
畜産草地研究所所長
松本　光人

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Somatic Cell Cloned Cattle and Their Progeny Produced in Japan : A Report for Animal Health and Characteristics of Animal Products

Shinya Watanabe

National Institute of Livestock and Grassland Science, NARO, Tsukuba, 305-0901 Japan

SUMMARY

This report reviews the Japanese domestic findings concerning animal health and characteristics of animal products on somatic cell cloned cattle and their progeny. These finding were accumulated by nationwide two surveys for these animals (carried out on April, 2005 and on July, 2006) and a research project supported by Ministry of Agriculture, Forestry and Fisheries, Japan. These surveys covers such findings as shown bellow; (1) Clinical examination, hematology and clinical chemistry for 63 somatic cell cloned cattle (about 60% of surviving clones at the time of the survey) and 25 progeny of clones, (2) Life time data of 482 somatic cell cloned cattle (97.5% of clones produced at the time of the survey) and 202 progeny of clones. Moreover, 74 Japanese-written papers describing animal health of somatic cell cloned cattle and their progeny were also collected. The findings, which accumulated by Japanese institutions employing 173 somatic cell cloned cattle (51.6% of clones produced at the time of a survey) and 31 progeny of clones, recorded in these reports were categorized as shown bellow; (1) Clinical and pathological findings (individual identification, hematology, clinical chemistry and pathology), (2) Growth performance, (3) Reproductive performance, (4) Milk/meat productive performances. By analyzing these findings obtained in Japan, it revealed that health status of somatic cloned cattle surviving more than 200 days after birth seems to be practically equivalent to those of conventional bred cattle. It would be also true in progeny of somatic cell cloned cattle throughout their all lifetime. With regard to findings concerning the characteristics of milk/meat derived from somatic cell cloned cattle and their progeny produced in Japan, no biologically significant differences in nutritional analysis, detection of anaphylactic reaction (mouse abdominal wall method), digestion test (in rats), micronucleous test (in mice) and feeding test (in rats) were observed when these findings were compared with those of conventionally bred cattle.

Keywords: cattle, somatic cell clone, progeny, animal health, animal product characteristics

1. Introduction

On November 1999, voluntary moratorium of somatic cell cloned cattle was demanded by Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan. After that time, some Japanese researchers had been investigated safety of food products derived from somatic cell cloned cattle. In 2003, the "Kumagai report (Titled: "Safety of animal products produced by cloning technology")" [29] was published. It was the most important Japanese document concerning safety of food products derived from somatic cell cloned cattle in this period.

The issues discussed in the "Kumagai report"[29] were as shown bellow; (1) Safety of cloned cattle as derivation of food product, (2) Development of animal reproduction technologies, (3) Concerns of

mitochondria in safety of animal products derived from somatic cell cloned cattle, (4) Nuclear imprinting and abnormal proliferation. As a conclusion of the discussion, any novel factors that affect safety of cloned cattle derived from somatic/embryonic cells could not found when these findings were compared to those obatined from conventionally bred cattle.

In 2008, official risk assessment reports on the food safety of food products derived from somatic cell cloned animals and their progeny were issued by the Food and Drug Administration (FDA) of the United States of America and the European Food Safety Authority (EFSA) [1,2]. These reports concluded that the risk of consuming milk/meat derived from somatic cell cloned animals (e.g. cattle and pig) and their progeny was practically equivalent to that in conventionally bred animals.

A purpose of the present report was to review Japanese nationwide data concerning animal health and characteristics of animal products on somatic cell cloned cattle and their progeny. Especially, this review concerning progeny of clones had been required for people who were thinking about practical use of somatic cell cloned cattle as breeding animals, since production cost of progeny of clones by artificial insemination (AI) seems to be equivalent to that of conventionally bred cattle. When the present report was prepared, issues in the "Kumagai report" [29] as shown below were taken into consideration; (1) To evaluate the benefit of somatic cell cloned cattle, carefully observation of these animals concerning growth, reproduction and physiology would be essential, (2) To investigate lifetime data of somatic cell cloned cattle including birth and death loss of these animals should be accumulated with nationwide scale.

The present report were based on two nationwide surveys (carried out on April, 2005 and July, 2006) and a research project "Development of production technology and investigation on somatic cell cloned cattle for practical use (project #1602, 2004-2008)" supported by a Grant-in Aid for a Research Project for Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries (UATAFF) from the Agriculture, Forestry and Fisheries Research Council, MAFF, Japan. In the project, the most important investigation was "Characteristics of milk/meat derived from progeny of somatic cell cloned cattle". The investigation was designed to complement of a three-year project (1999-2002) concerning the characteristics of milk/meat

Table 1. Research fields of data collected in previous risk assessment reports (PRAS) and in the present report (PR)

Developmental node defined by FDA		Kinds of animals	Research fields											
			① Production and lifetime		② Clinical and pathological investigations		③ Growth performance		④ Reproductive performance		⑤ Milk/meat productive performance		⑥ Characteristics of animal products	
Node	Developmental stage		Birth weight, gestation period, age of death, causes of death		Hematology, clinical chemistry, pulse rate, rectal temperature, pathology		Body weight, withers height		Fertility, endocrinology		Milking, milk yield, body weight gain, carcass traits, meat quality, composition analysis		Toxicity test, detection of anaphylactic reaction, detection of mutagenicity	
			PRAS	PR	PRAS	PR	PRAS	PR	PRAS	PR	PRAS	PR	PRAS	PR
1	Pregnancy and parturition	Clones	NDA	NDA	K, F	NDA								
		Progeny	NDA	R	NDA	NDA								
2	Perinatal period	Clones	K	N	K, F	N, R	K	R						
		Progeny	NDA	N	NDA	N,P, R	NDA	R,P						
3	Juvenile development	Clones	K	N	K, F	N, R	K, F	R						
		Progeny	NDA	N	NDA	N,P	NDA	R,P						
4	Reproductive development and function	Clones	K	N	K, F	N, R	NDA	R	K, F	L, P				
		Progeny	NDA	N	NDA	N,P	NDA	R	NDA	P				
5	Post-pubertal maturation and aging	Clones	K	N	K, F	N, R	F	R	K, F	R	K, F	R	K, F	NDA
		Progeny	NDA	N	NDA	N,P, R	NDA	R	NDA	P	F	P	NDA	P

Previous risk assessment reports; K: Kumagai report (2003), F: Risk assessment report by FDA (2008)
Events of data collection for the present reports; P: Project for Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries (2007), N: Nationwide survey by NILGS (2005, 2006), R: Reference survey by NILGS(2006), L: Local investigation by NILGS (2006)
NDA: No data available

derived from embryonic/somatic cell cloned cattle by the Japan Livestock Technology Association, " Investigation on the attributes of cloned bovine products" [14].

The present report would include instructive finding for risk analysis of animal products derived from somatic cell cloned cattle and their progeny, since objectively reliable findings are required for the risk analysis [33].

2. Investigation concerning animal health status and characteristics of animal products on somatic cell cloned cattle and their progeny conducted in Japan

The first application of cloning technology to domestic animals was achieved in 1986 as a production of embryonic cloned sheep by Willadsen[69]. After ten years attempt of cloning studies, Wilmut *et al*. produced a somatic cell clone sheep, Dolly in 1997 [70]. At that time, there were many researchers and technical experts who had advanced technique concerning embryo transfer (ET), embryo manipulation, *in vitro* fertilization and oocyte/embryo culture in Japan. Of such talented Japanese, Kato *et al*. succeeded in producing the first somatic cell cloned cattle derived from adult animal on July 5, 1998 [21]. Following their success, hundreds of somatic cell cloned cattle have been produced by many Japanese institutions including local experimental stations, which were established by local governments.

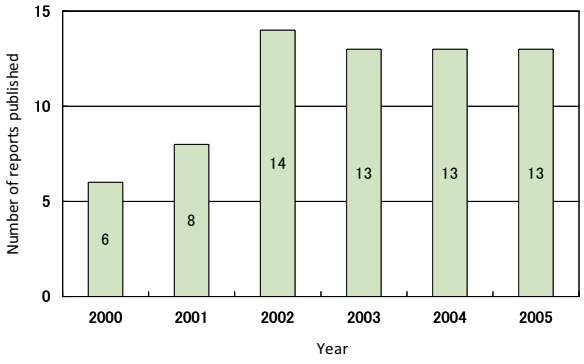

Fig. 1. Number of reports concerning somatic cell cloned cattle and their progeny published in Japan

2.1 Reports concerning animal health of somatic cell cloned cattle and their progeny published in Japan

Although many investigations employing somatic cell cloned cattle and their progeny have been carried out by Japanese institutions, small number of animals in each investigation prevented elegant experimental design, which major international journals demand as a scientific report. Therefore, most of precious findings obtained from these animals have been published in institution bulletin or domestic journal written in Japanese language. Unfortunately, it is hard to read these reports even Japanese scientist, since such reference cannot find in database search such as PubMed.

To obtain nationwide data for reviewing health status of somatic cell cloned cattle and their progeny, a survey for domestic reports was carried out in July, 2006. As a result, 74 such reports describing health status of somatic cell cloned cattle and their progeny could be obtained with cooperation of Japanese institutions [66,67]. Of these domestic report obtained, the first report concerning somatic cell cloned cattle was published in 2000, only two years after the first production of somatic cell cloned cattle in Japan, and number of published reports reached to 14 in 2002 (Figure 1). It showed aggressive reach activity concerning animal cloning at that time of Japan. These reports included in 14 institution bulletins, 14 project reports, 10 domestic journals, five supplements and one other booklet. It should be noted that 59.6% of reports included in institution bulletins.

2.2 Number of somatic cell cloned cattle and their progeny employed for investigations concerning their health status

To find combined total number of somatic cell cloned cattle and their progeny investigated for animal health status by Japanese institutions, the 74 domestic reports were analyzed. As a result, it revealed that 173 somatic cell cloned cattle and 31 progeny of clones in actual number were employed by Japanese institutions. Since some animals were used in plural investigations, the actual number of clones and their

progeny were 253 and 37, respectively. Although the number of animals used in each report was small, total number of animals was more than ten in some investigation categories when the number of animals was accumulated in a nationwide scale (Table 2, 3). To increase the number of animals, additional progeny of clones were employed in the UATAFF project #1602 (Table 3).

2.3 Elucidation for characteristics of animal products derived from somatic cell cloned cattle and their progeny

The only investigation concerning characteristics of animal products derived from somatic cell cloned cattle was carried out by Research Institute for Animal Science in Biochemistry and Toxicology (RIAS), which received confirmation of GLP (Good Laboratory

Table 2. Number of somatic cell cloned cattle employed for each research field in the present report

Breed	Sex	Research fields							Number of cattle			
		Clinical and pathological investigations			Growth performance	Reproductive performance	Milk/meat productive performance		Total number (with overlapping)	%	Actual number (without overlapping)	%
		Individual identification	Hematology and clinical chemistry	pathology			Milk	Meat				
Japanese Black beef cattle	♂	21	19	7	24	16	0	14	101	40 1	77	44 5
	♀	14	7	5	15	9	2	5	57	22 6	40	23 1
Holstein dairy cattle	♂	0	0	0	0	0	0	0	0	0 0	0	0 0
	♀	16	4	3	18	19	16	0	77	30 2	46	26 6
Jersey dairy cattle	♂	0	0	0	0	0	0	0	0	0 0	0	0 0
	♀	0	0	0	4	4	4	0	12	4 8	4	2 3
Japanese Brown beef cattle	♂	0	0	0	0	2	0	0	2	0 8	2	1 2
	♀	0	0	0	0	2	0	0	2	0 8	2	1 2
F_1	♂	2	0	0	0	0	0	0	2	0 8	2	1 2
	♀	0	0	0	0	0	0	0	0	0 0	0	0 0
Number of cattle (with overlapping)	Total	53	30	15	61	52	23	19	253	100 0	173	100 0
	%	21 0	11 9	6 0	24 2	20 6	8 7	7 5	-	-	-	-

Table 3. Number progeny of somatic cell cloned cattle employed for each research field in the present report

Breed	Sex	Research fields																				Total number of cattle							
		Clinical and pathological investigations									Growth performance			Reproductive performance			Milk/meat productive performance						With overlapping			%	Without overlapping (Actual number)			%
		Individual identification			Hematology and clinical chemistry			pathology									Milk			Meat										
		R	P	Total	R	P	Total	R	P	Total	R	P	Total	R	P	Total	R	P	Total	R	P	Total					R	U	Total	
Japanese Black beef cattle	♂	0	0	0	0	4	4	0	0	0	0	4	4	0	0	0	0	0	0	8	0	8	8	8	16	21.3	8	4	12	25.5
	♀	0	0	0	2	2	4	1	0	1	1	2	3	0	0	0	0	0	0	10	4	14	14	8	22	29.3	13	6	19	40.4
Holstein dairy cattle	♂	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	2.7	1	0	1	2.1
	♀	0	0	0	4	5	9	1	0	1	3	5	8	0	5	5	0	5	5	0	0	0	8	20	28	37.3	4	5	9	19.1
Jersey dairy cattle	♂	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0	0	0	0.0
	♀	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0	0	0	0.0
Japanese Brown beef cattle	♂	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	1.3	1	0	1	2.1
	♀	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	4	4	0	4	5..3	4	0	4	8.5
F_1	♂	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0	0	0	0.0
	♀	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	2	2	2.7	0	1	1	2.1
Number of cattle (with overlapping)	計	0	0	0	7	15	22	3	0	3	4	16	20	0	5	5	0	5	5	23	5	28	37	38	75	100.0	31	16	47	100.0
	%	0.0	0.0		18.9			8.1			10.8			0.0			0.0			62.2			-			-	-			-

Events of data collection for the present reports; R: Reference survey by NILGS (2006), P: Project for Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries (2007).

Practice), as a three-year project (1999–2002) from the Japan Livestock Technology Association. In this project, the following data obtained from milk/meat were compared between embryonic/somatic cell cloned cattle and conventionally bred cattle: general components, amino acids and fatty acids, digestibility, allergenicity (in mice), mutagenicity (in mice) and fourteen-week feeding test (in rats). In the project, tree of each embryonic cell cloned cows, somatic cell cloned cows and conventionally bred cows were employed for producing milk. For meat production, one embryonic cell cloned steer, one somatic cell cloned steer and three conventionally bred steers were fattened up. The detailed data could be found as a Japanese language written report [14].And also a part of the report could found in the risk assessment report of FDA as "Appendix I", which translated in English [1].

With regard to characteristics of animal products derived from progeny of somatic cell cloned cattle, there were no available data at the period of the "Kumagai report" [29]. Therefore, "progeny version" of the investigation shown above was designed and commissioned to RIAS by the National Institute of Livestock and Grassland Science (NILGS) as a part of the UATAFF project #1602.

3. Findings concerning animal health of somatic cell cloned cattle and their progeny obtained in Japan

3.1 Production and their lifetime

3.1.1 Press release by MAFF.

In the case of Japan, MAFF have been accumulated nationwide data concerning production and lifetime of somatic cell cloned cattle since 1999. Current status of production and death/slaughter was disclosed by monthly (flash data) and half-yearly (confirmed data) press release of MAFF. These data written in Japanese language could obtain in web page of MAFF (www. affrc. go.jp).

According to the press release on October 31, 2007, 535 somatic cell cloned cattle was produced in Japan since July 5, 1998 (birthday of the first somatic cell cloned cattle produced in Japan). The major breeds of clones were Japanese Black beef cattle and Holstein dairy cattle. These clones were produced by 40 institutions including 33 local experimental stations.

3.1.2 Production and death/slaughter

To obtain lifetime data including production and death/slaughter of somatic cell cloned cattle and their progeny, a nationwide survey was carried out by NILGS in August, 2006. As a result, lifetime data concerning 482 somatic cell cloned cattle and 202 progeny of clones were submitted by 39 Japanese institutions. All of these somatic cell cloned cattle were non- genetically modified animals. According to press release of MAFF at that time, 495 somatic cell cloned cattle were produced. Therefore, it could be estimated that the lifetime data would cover 97.3% of somatic cell cloned cattle produced at the time of a survey.

1) Production

It is confirmed that the first somatic cell cloned cattle were produced on July 5, 1998 by the present survey. Within the same year, 31 somatic cell cloned cattle were produced in Japan (Figure 2). Additional 81 somatic cell cloned cattle were produced in the next year; however, the production declined to 53 in 2005.

With regard to progeny of somatic cell cloned cattle, the first animal in Japan was produced on July 10, 2000. Twenty-one progeny were produced in the same year. Although 46 progeny were also produced in the subsequent year, the number had been decreased to 25 on 2005 (Figure 2). Among 202 progeny of cloned cattle produced in Japan, 95.0% (192/202) were produced by AIs and 5.0% (10/202) by ETs. The details

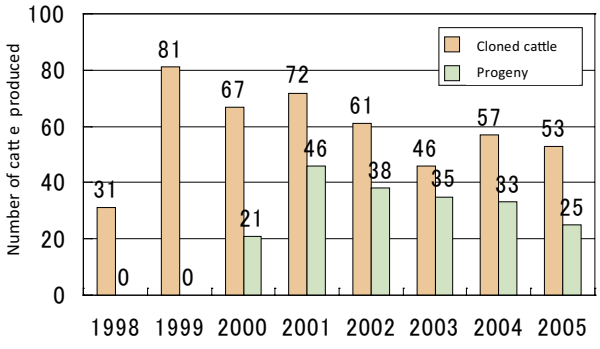

Fig. 2. Number of somatic cell cloned cattle and their progeny produced in Japan

of the progeny production system are as follows: (1) AI to conventionally bred cow with semen derived from somatic cell cloned bull (n=63), (2) AI to somatic cell cloned cow with semen derived from conventionally bred bull (n=115), (3) AI to somatic cell cloned cow with semen derived from somatic cell cloned bull (n=7), (4) ET to conventional bred cow with embryo produced from system (2) (n=3), (5) ET of Somatic cell cloned cow with embryo produced from system (2) (n=3), (6) ET to somatic cell cloned cow with embryo produced from system (3) (n=3) . Two ground daughters/sons of somatic cell cloned cattle were also produced.

Among 482 somatic cell cloned cattle produced, 78.8% (380/482) were Japanese black beef cattle and 15.5% (75/482) were Holstein dairy cattle (Figure 3). With regard to 202 progeny of clones, 44.6% (90/202) were Japanese black beef cattle and 32.2% (65/202) were Holstein dairy cattle. The ratios of females in somatic cell cloned cattle and their progeny were 51.4% (248/482) and 52.5% (106/202), respectively. No male Holsteins were produced in somatic cell cloned cattle.

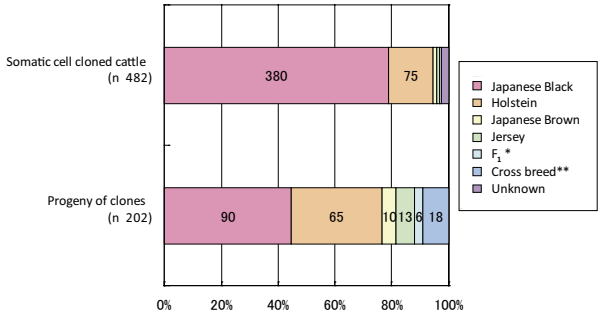

Fig. 3. Breeds of somatic cell cloned cattle and their progeny produced in Japan
* Japanese Black × Holstein, **Excepting F_1.

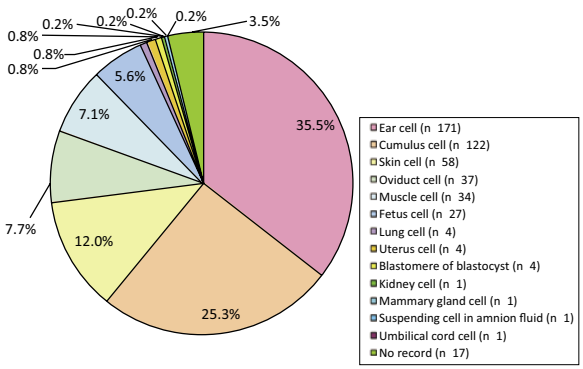

Fig. 4. Sources of donor cells used for cattle cloning in Japan (n=482, all cloned cattle surveyed)

2) Donor cells and recipient oocytes

The major sources of donor cells were the ear (35.5%; 171/482), cumulus (25.3%; 122/482), skin (12.0%; 58/482) and oviduct (7.7%; 37/482) (Figure 4). Almost all recipient oocytes, which were used for nuclear transfer, were derived from ovaries collected at slaughterhouses.

3) Death and slaughter

i) Death losses due to stillbirth and neonatal death

Of all cloned cattle investigated, the death loss due to stillbirth in calves were 16.4 (79/482) and 8.9% (18/202) for somatic cell cloned cattle and their progeny, respectively (Figure 5). In Japanese Black beef cattle and Holstein dairy cattle that could obtain data concerning conventionally bred cattle, the significant differences were analyzed among somatic cell cloned cattle, their progeny and conventionally bred cattle in these breeds. As a result, the death loss due to stillbirth in calves were 16.4 (74/451), 8.9 (11/124) and 4.6 % (26/566) for somatic cell cloned cattle, their progeny and conventional bred cattle, respectively (Figure 6). Significant differences in death losses due to stillbirth were observed between somatic cell cloned cattle and their progeny ($P<0.05$), and somatic cell cloned cattle and conventionally bred cattle ($P<0.01$); however, there was no significant difference between the progeny and conventionally bred cattle.

With regard to death loss due to neonatal death (death within 24 hours) in calves were 14.4% (65/451), 0.8% (1/124) and 1.9% (11/566) for somatic

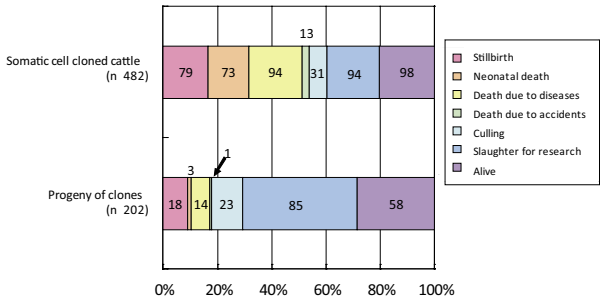

Fig. 5. Status of somatic cell cloned cattle and their progeny at the time of a survey (All cattle surveyed)

cell cloned cattle, their progeny and conventional bred cattle, respectively (Figure 6). Significant differences in death losses due to neonatal death of calves were observed between somatic cell cloned cattle and their progeny (P<0.01), and somatic cell cloned cattle and conventionally bred cattle (P<0.01); however, there was no significant difference between the progeny of somatic cell cloned cattle and conventionally bred cattle.

According to data sheets from the survey, incidences of respiratory problems in somatic cell cloned cattle were 16.7 (8/48) and 50.7% (35/69) for stillbirth and neonatal death, respectively.

A tendency of large offspring syndrome was observed in the cases of neonatal death. Namely, in Holstein dairy cattle, the birth weights of cloned newborns were 53.6±11.2 (n=16, mean±SD), 44.5±10.4kg (n=9) for neonatal death and alive cases. The reference data of birth weight of Holstein dairy cattle in the same time point was 40.5±5.8kg (n=137). In the case of progeny of clones, such comparison could not performed due to only three newborn data.

In conclusion, these findings show that the death losses due to stillbirth and neonatal death in somatic cell cloned cattle are higher than those of conventionally bred cattle; however, the losses in the progeny are in the same level observed in conventionally bred cattle.

ii) Slaughters for investigation

The numbers of slaughtered cattle for investigation of carcass and/or organs were 94 and 85 for somatic cell cloned cattle and their progeny, respectively (Figure 5). When the slaughtered case in Japanese Black beef cattle and Holstein dairy cattle were accumulated, the most of slaughters were carried out after 500 and 800 days of age for somatic cell cloned cattle and their progeny, respectively (Figures 7, 8-1 and 8-2). The main purposes of slaughters were investigations of carcass characteristics after fattening trial and autopsy (Figure 9). After the fattening trial, the percentage of A5 grading dressed meat (the highest quality in Japanese meat grading) was 51.0% (26/51) and 27.5% (11/40) for somatic cell cloned cattle and their progeny, respectively. The high frequency of A5 grading meat would be due to selection of nuclear donor; cattle in excellent pedigrees were often chosen as nuclear donors. In the cases of autopsy, incidence of no specific findings were found in 78.7% (34/47) and 96.7% (58/60) for somatic cell cloned cattle and their progeny, respectively. It should be noted that 35.7% (10/28) of male progeny in Holstein dairy cattle were slaughtered just after birth, since it was hard to find out any targets of investigation concerning milking. Therefore, high incidence of slaughters was observed in Figure 8-2, which accumulated with both sexes of the progeny in Japanese Black beef cattle and

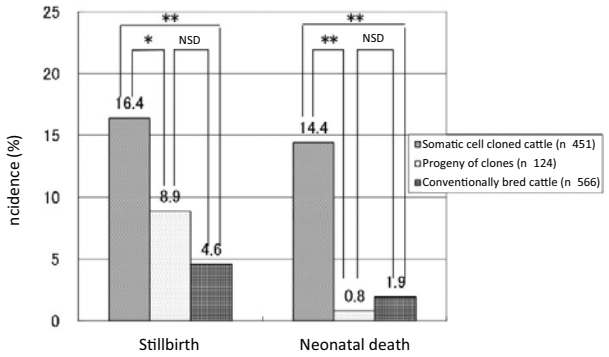

Fig. 6. Death losses due to stillbirth and neonatal birth in somatic cell cloned cattle, their progeny and conventionally bred cattle. These data focused on Holstein (female) and Japanese Black (both sexes) due to the composition of available conventionally bred cattle breeds. *: P<0.05, **: P<0.01, NSD: Not significant difference (by χ^2 test).

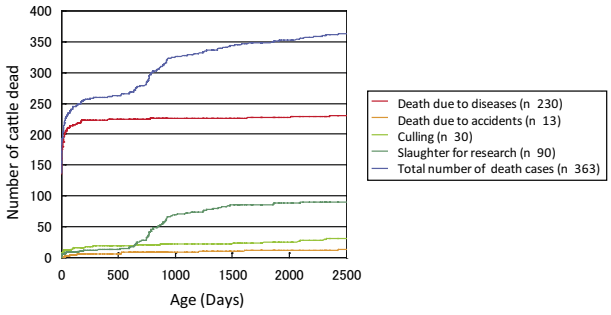

Fig. 7. Accumulation of slaughters for research in somatic cell cloned cattle produced in Japan. The data focused on Holstein (female) and Japanese Black (both sexes) due to available control data of conventionally bred cattle breeds. Stillbirths and neonatal deaths are including to “death due to diseases”.

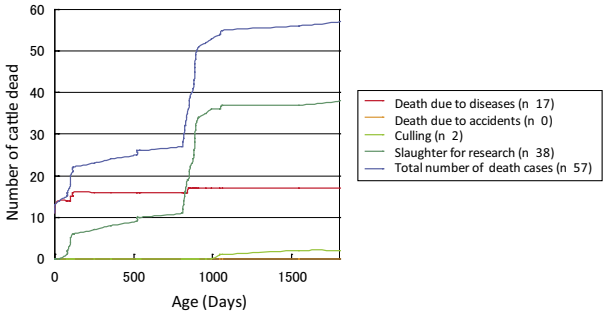

Fig. 8-1. Accumulation of slaughters for research in progeny of clones produced in Japan. The data focused on Holstein (female) and Japanese Black (both sexes) due to available control data of conventionally bred cattle breeds. Stillbirths and neonatal deaths are including to “death due to diseases”.

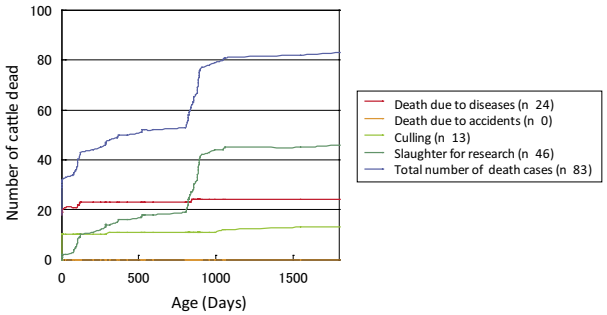

Fig. 8-2. Accumulation of slaughters for research in progeny of clones produced in Japan. The data focused on Holstein (both sexes) and Japanese Black (both sexes) for composition with Fig. 8-1. High frequency of culling just after birth was observed due to valueless of Holstein males. Stillbirths and neonatal deaths are including to “death due to diseases”.

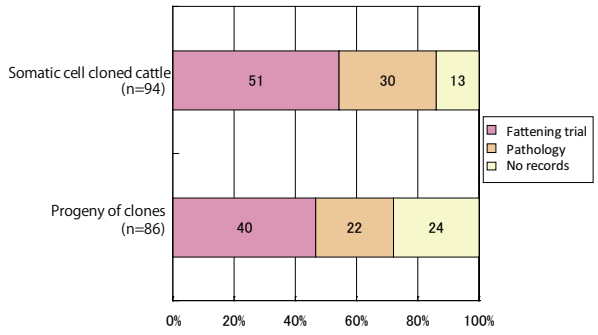

Fig. 9. Main purposes of slaughters for research in somatic cell cloned cattle and their progeny produced in Japan (All cattle surveyed)

Holstein dairy cattle, when it is compared with Figure 8-1, which accumulated with both sexes of the progeny in Japanese Black beef cattle and female progeny of Holstein dairy cattle.

iii) Death losses due to diseases

Of all cattle investigated, the incidence of death losses due to diseases were 19.5% (94/482) and 6.9% (14/202) for somatic cloned cattle and their progeny, respectively (Figure 5). Death losses due to diseases in somatic cell cloned cattle, their progeny and conventionally bred cattle were analyzed in every 30-days after birth in Japanese Black beef cattle and Holstein dairy cattle, since control data could obtain in these breeds. With regard to somatic cell cloned cattle, although a higher death loss due to diseases (24.1%, 52/216) was observed in the first 30 days of their life, the loss reached the same level as that of conventionally bred cattle on about 200 days of age; however, incidence of death loss in the progeny was the same level as that of conventional bred cattle throughout their lifetime (Figure 10).

According to data sheets from the survey, the major observations of dead somatic cell cloned cattle during 2-3 days after birth were respiratory problems (35.3%; 6/17) and deformed hearts (11.8%; 2/17). After four days or later of birth, the major cause of dead somatic cell cloned cattle was pneumonia.

The death loss due to diseases was also examined by dividing the lifetime into 2-150, 150-300 and 300-720 days after birth (Figure 11). The age of 150, 300 and 720 days could be considered as important points in the lifetime of Japanese cattle. Namely, at 150 days of age, it has been considered that functions and the comparative volume of the rumen have matured in calves. At 300 days of age, many beef cattle would be on the calf market in Japan. At 720 days of age, beef cattle would start arriving at the slaughterhouse for meat production, and dairy cows could calve to produce milk. At 2-150 days of age, the incidence of death loss due to diseases were 23.5 (72/307), 4.5 (5/111) and 4.3% (55/1289) for somatic cell cloned cattle, their progeny and conventional bred cattle, respectively. Significant differences in the death loss were observed between somatic cell cloned cattle and

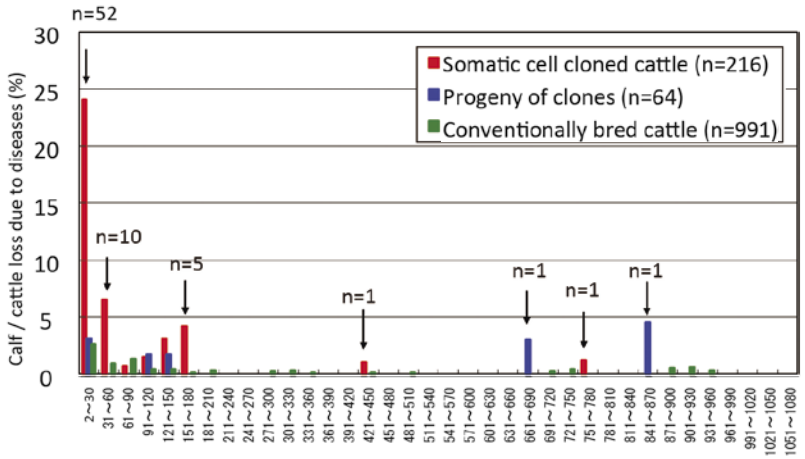

Fig. 10. Incidence of death loss due to diseases in from somatic cell cloned cattle, their progeny and conventionally bred cattle. Lifetime of cattle was divided into 30 days. These data focused on Holstein (female) and Japanese Black (both sexes) due to the composition of available conventionally bred cattle breeds. Death loss in a 30-days period was calculated as "Death loss (%) = (number of animals dead due to diseases /numbers of animals on the first of 30 days) x 100 (%)".

their progeny (P<0.01), and somatic cell cloned cattle and conventionally bred cattle (P<0.01); however, there was no significant difference in the loss between the progeny and conventional bred cattle. At 150-300 days of age, the incidence of death loss due to diseases were 2.5 (5/202), 0 (0/94) and 0.5 % (6/1207) for somatic cell cloned cattle, their progeny and conventionally bred cattle, respectively. Significant differences in the death loss were observed between somatic cell cloned cattle and conventionally bred cattle (P<0.01). In regard to 300-720 days of age, the incidences of the death loss in somatic cell cloned cattle, their progeny and conventionally bred cattle were within the same level.

These findings suggest that young beef cattle in the calf market (at 300 days old), fattened beef cattle in the slaughterhouses (>720 days old) and milking dairy cattle after first parturition (>720 days old) would show the same robust health as conventionally bred cattle.

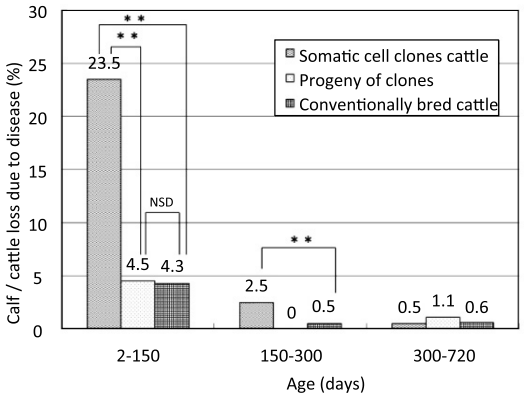

Fig. 11. Death loss due to diseases in somatic cell cloned cattle, their progeny and conventionally bred cattle. Lifetime of cattle was divided as 2–150, 150–300 and 300–720 days after birth, since the age of 150, 300 and 720 days could be considered as important points in the lifetime in cattle (see text in detail). These data focused on Holstein (female) and Japanese Black (both sexes) due to the composition of available conventionally bred cattle breeds. *: P<0.05, **: P<0.01, NSD: Not significant differnce (by χ^2 test).

4) Usages of living cattle at institutions

Numbers of Somatic cell cloned cattle and progeny of clones that surviving at the time of survey were 98 and 58, respectively (Figure 5). They were

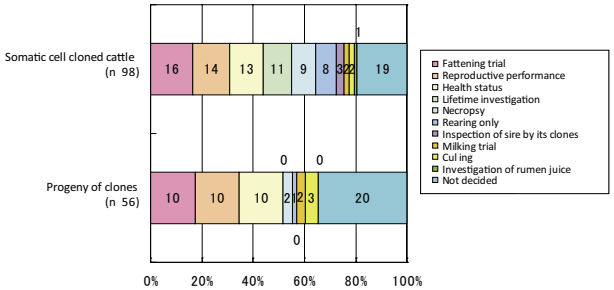

Fig. 12. Main purposes of research in somatic cell cloned cattle and their progeny rearing in Japan (All cattle surveyed)

mainly used for research including fattening trial, reproductive function and health status (Figure 12).

3.2 Clinical and pathological investigations

3.2.1 Individual identification

Genetic similarities among somatic cell cloned animals and their nuclear donors could be assumed by similar pattern of marking on their furs [40] (Figure 13). To assess similarity in these animals, investigations concerning individual identification were carried out with 53 clones (Table 2). Comparisons of muzzle prints were applied to four clone-nuclear donor sets that contained 13 somatic cell cloned cattle and four nuclear donors [6,9,12,37]. The muzzle print patterns exhibited conformity based on donor type; however, there was enough variance in pattern detail to discriminate between individuals (Figures 14, 15). Confirmation of genuine clones has been also conducted using 17-23 microsatellite polymorphism markers in 14 clone-nuclear donor sets that contained 40 cloned cattle[11,20,55,61,76]. No inconsistencies were found in any of the animal sets analyzed. The results show that clones and their nuclear donors have the same genetic traits.

It should be noted that no contradictions were found in the parent-child relationship among the four progeny of clones and two nuclear donors of their parents, when parentage diagnosis was carried out among these cattle (MIC Co. Ltd., unpublished data).

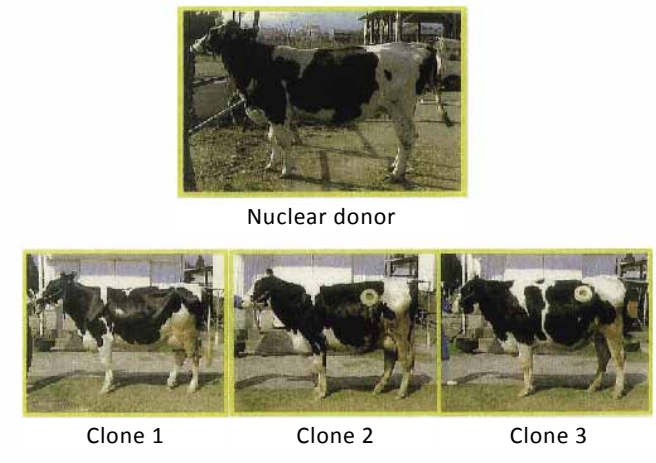

Fig. 13. Fur pattern observed in three somatic cell cloned cattle and their nuclear donor (Holstein dairy cattle, cited from reference #40 with permission)

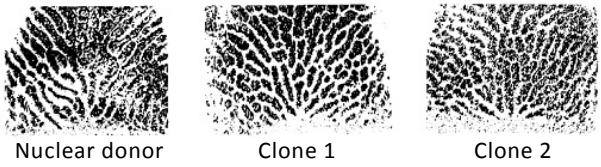

Fig. 14. Nuzzle print pattern observed in two somatic cell cloned cattle and their nuclear donor (Japanese Black beef cattle, cited from reference #9 with permission)

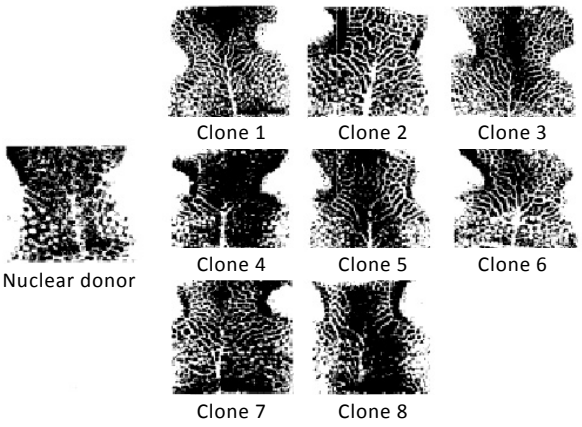

Fig. 15. Nuzzle print pattern observed in eight somatic cell cloned cattle and their nuclear donor (Holstein dairy cattle, cited from reference #37 with permission)

3.2.2 Hematology and clinical chemistry

Thirty somatic cell clones were subjected to "hematology and clinical chemistry" examinations [4,9,19,28,39,41,62,78] (Table 2). All investigations were performed in cattle within 12 months after birth.

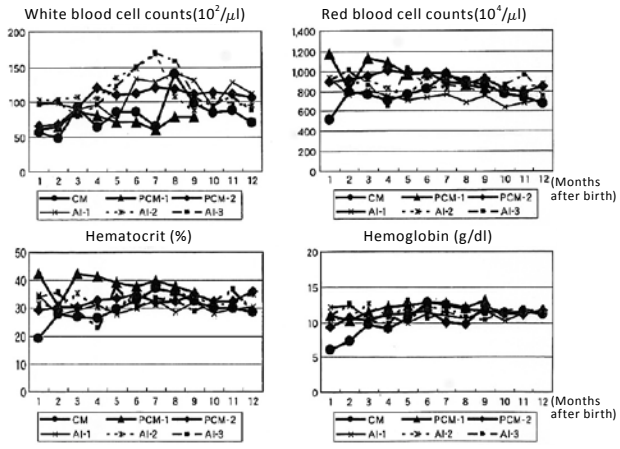

Fig. 16. Changes in hematological parameters observed in a somatic cell cloned cattle (CM), its progeny (PCM-1, 2) and conventionally bred cattle (AI-1, 2, 3) (Males, Japanese Black beef cattle, cited from reference #4 with permission)

According to the classification of developmental stages defined by the FDA [1], these animals covered the stages from Developmental Node 2 (perinatal period) to Developmental Node 4 (reproduction development and function node). The state of newborns health during the first month was the greatest concern of these investigations. One to 14 hematological parameters, including red blood cell count (RBC), white blood cell count (WBC) and hematocrit, have been examined. The clinical chemistry data covered five to 42 parameters, including glucose, blood urea nitrogen (BUN) , lactate dehydrogenase (LDH) and calcium. The values obtained from the clones surviving to adulthood seemed to be within the range of variation for individuals (Figures 16, 17). No remarkable differences between clones/progeny and conventionally bred cattle were found in these observations.

With regard of progeny of clones, seven cattle were investigated [4,19,39,73] (Table 3). The developmental Nodes were 2-4. These investigations were also performed in cattle within 12 months after birth. Two to 14 hematological parameters, including RBC, WBC and hematocrit, have been examined. The clinical chemistry data covered six to 24 parameters, including glucose, BUN, LDH and calcium. Although significant differences in these parameters were found, no parameters showed gross deviation from those obtained from conventionally bred cattle (Figures 16, 17). Similar results were obtained in 11 progeny of cloned cattle for 7-9 hematological parameters and 17-24 clinical chemistry parameters as a part of the UATAFF project #1602 (unpublished data).

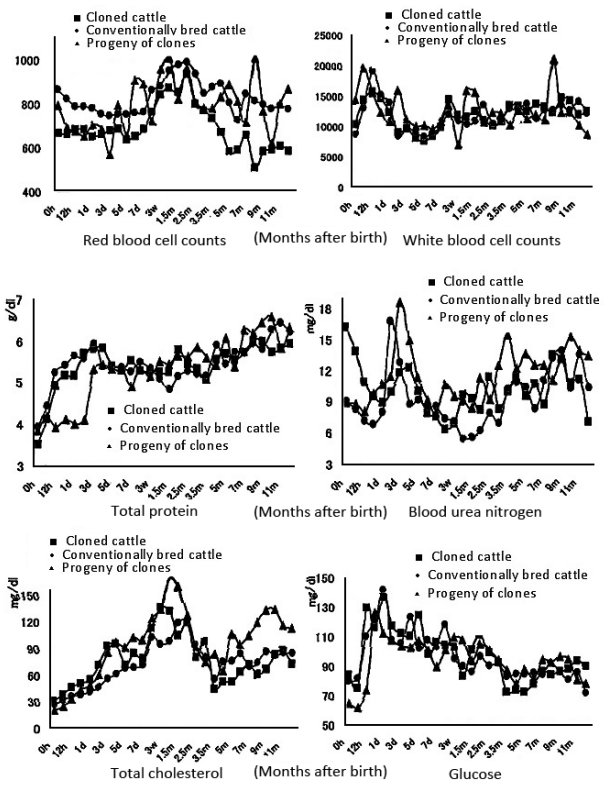

Fig. 17. Changes in clinical chemistry parameters observed in a somatic cell cloned cattle, its progeny and conventionally bred cattle (Females, Holstein dairy cattle, cited from reference #39 with permission)

3.2.3 Pathology

Fifteen cloned cattle were used for pathological investigations [4,31,72,78] (Table 2). The observed Developmental Nodes of the animals were 2 (perinatal period), 3 (juvenile developmental node) and 5 (post-puberty maturation and aging). Of these clones, one third (4/13) were dead newborns in Developmental Node 2. Most animals observed in this Node possessed abnormalities of the heart, lung, kidney and umbilical cord. With regard to Developmental Nodes 3 and 5, no anomalies were found in any of the 6 clones, which seemed to be healthy when sacrificed for observation. In conclusion, the somatic cell cloned cattle that were healthy in appearance exhibited hardly any abnormalities in pathological observations; however, a large number of the dead animals possessed lethal abnormalities. It should be noted that the causes of death for the clones were well-known diseases in conventionally bred cattle.

Three progeny were used for pathological investigations [4,73] (Table 3). In a case of stillbirth, immunodeficiency, which had been observed in conventionally bred cattle, was found [72]. Any abnormal pathological findings were not observed in two healthy adults.

3.2.4 Nationwide survey for clinical investigation (on April 2005)

To obtain clinical data concerning animal health status on somatic cell cloned cattle and their progeny produced in Japan, a nationwide survey was carried out on April, 2005 with cooperation of institutions that produced somatic cell cloned cattle.

As a result, clinical data (body weight, respiratory rate, pulse rate and rectal temperature) and blood samples from 63 somatic cell cloned cattle, 25 progeny of clones and 83 conventionally bred cattle and two other cattle including grand children of clones were submitted from 21 institutions. Hematological parameters (RBC, WBC, hemoglobin, hematocrit) and clinical chemistry parameters (glutamic-oxaloacetic transmainase (GOT), glutamic-pyruvic transaminase (GPT), γ-glutamyltranspeptidase (γ-GTP), BUN, total bilirubin, total protein, glucose, uric acid, neutral fat, alkaline phosphtase (ALP), creatinine, total cholesterol, albumin, creatinine phosphokinase (CPK), leucine aminopeptidase (LAP), inorganic phosphorous, magnesium,calcium, amylase, LDH, sodium (Na), potassium (K) and chlorine (Cl) were obtained by analyzing blood samples submitted from institutions. The data obtained data shown above covered 60.6% (63/104) of somatic cell cloned cattle produced at the point of the survey. No significant differences or abnormalities in these parameters obtained from somatic cell cloned cattle and their progeny were found when theses were compared with those from conventionally bred cattle (NILGS, unpublished data).

3.3 Growth Performance

Sixty-one somatic cell cloned cattle were employed for growth performance investigations [3,6,9,11-13,15,16,19,20,30,41,42,44,45,53-55,58,61,63,64,72,74,77] (Table 2). The observation period covered the stages from Developmental Node 2 (perinatal period) to Developmental Node 5 (post-puberty maturation and aging). One to twelve growth performance parameters including body weight, withers height, heart girth and chest depth were investigated. When a nuclear donor was looked after contracted farmer, an institution might not obtain growth performance data from the farmer; therefore, the developmental data of clones could not compared with those obtained from nuclear donor. In such case, data obtained from conventionally bred cattle or standard growth curves issued by cattle registry association were compared with those obtained from clones. These findings demonstrated that the growth curves observed in somatic cell cloned cattle surviving to adult food showed similar increase when these were compared with those obtained from conventionally bred cattle (Figures 18–20). And the growth speed of clones was in a range of reference data issued by cattle registry association. It should be noted that high growth performance of a nuclear donor inherited to somatic cell cloned cattle derived from the donor (Figure 18).

Four progeny of clones were employed for growth

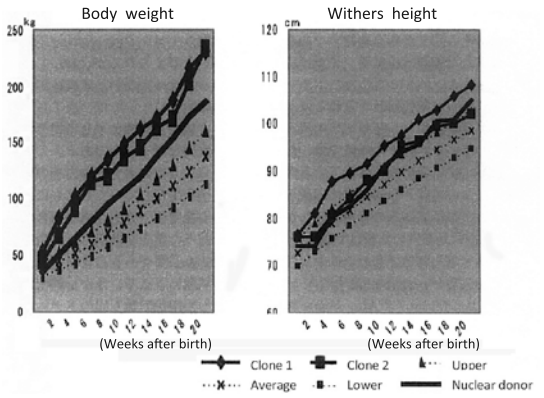

Fig. 18. Growth curves observed in a somatic cell cloned cattle and its nuclear donor
(Males, Japanese Black beef cattle, cited from reference #3 with permission)

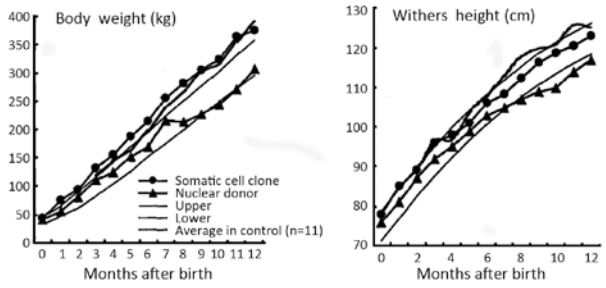

Fig. 19. Growth curves observed in a somatic cell cloned cattle and its nuclear donor
(Females, Holstein dairy cattle, cited from reference #16 with permission)

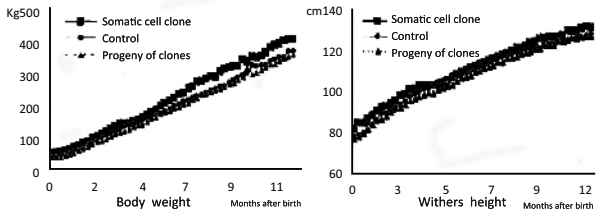

Fig. 20. Growth curves observed in somatic cell cloned cattle (n=3) and their progeny (n=3)
(Females, Holstein dairy cattle, cited from reference #39 with permission)

performance investigations [18,20,39] (Table 3). The Developmental Nodes during the observation period were Nodes 2 (perinatal period) to 4 (reproductive development and function node). One to four growth parameters body weight, withers height, chest girth and chest depth were also measured during the first year after birth. As a result, the growth performance of progeny of clones surviving to adult food was equivalent to conventionally bred cattle (Figures 18–20). Similar findings were also obtained in 11 progeny investigated as a part of the UATAFF project #1602 (Oita Prefectural Agriculture, Forestry and Fisheries Research Center, and National Livestock Breeding Center (NLBC), unpublished data).

3.4 Reproductive Performance

All investigations in reproductive performance, which consisted of 18 cloned bulls and 34 cloned cows [5,7,10,12,15-17,25-27,31,32,34,35,38,52,53,55,58,60,63-65,73,77,79] (Table 2), were in Developmental Node 4 (reproductive development and function node). In somatic cell cloned bulls, they produce normal semen after they reached puberty. These bulls exhibited normal semen characteristics, such as sperm concentration, sperm motility and semen pH (Table 4, Figure 21).

Table 4. Semen property of somatic cell cloned bull and its nuclear donor

	Somatic cell cloned cattle	Nuclear donor
Age (Months)	13	15
Volume (ml)	7.3	3.2
pH	7.0	6.6
Sperm motility, fresh +++ (%)	75	60
Spam concentration (10^8/ml)	10.6	8.3
Abnormal morphology (%)	8.5	7.9
Sperm motility, after freeze and thawing +++ (%)	35	20

(Japanese Black beef cattle, cited from reference #26 with permission)

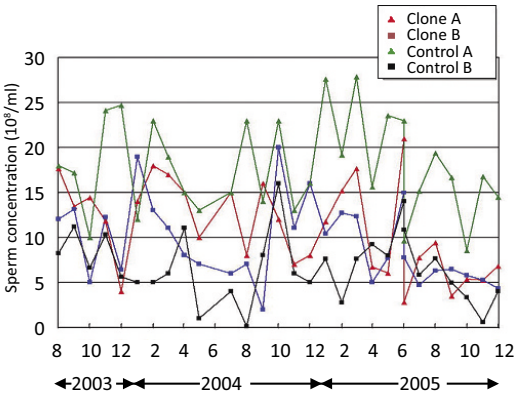

Fig. 21. Changes in sperm concentration observed in somatic cell cloned bulls (n=2) and conventionally bred bulls (n=2)
(Japanese Black beef cattle, cited from reference #68 with permission)

Table 5. *In vitro* fertilization with semen produced by somatic cell cloned bull and its nuclear donor

Name of sires	Number of oocytes inseminated	Number of oocytes cleaved after insemination	Cleavage rate (%)	Number of oocytes developed to blastocyst stage	Blastosyst formation rate (%)
NT1	537	407	75.8	191	35.6
NT2	636	500	78.6	252	39.6
MITSUSHIGE-ET*	120	91	75.8	41	34.2

*Nuclear donor of NT1 and NT2

(Japanese Brown beef cattle, cited from reference #60 with permission)

Table 6. Artificial insemination with semen produced by somatic cell cloned bull and its nuclear donor

Sire	Number of cows artificially inseminated	Pregnancy rate (%)	Abortion rate (%)	Number of newborns delivered	Calf production rate
Control	15	9 (60)	3 (20)	6	40%
Nuclear donor	7	3 (43)	1 (14)	2	29%
Clone 1	12	8 (67)	2 (25)	6	50%
Clone 2	12	6 (50)	1 (17)	5	42%

(Japanese Black beef cattle, cited from reference #64 with permission)

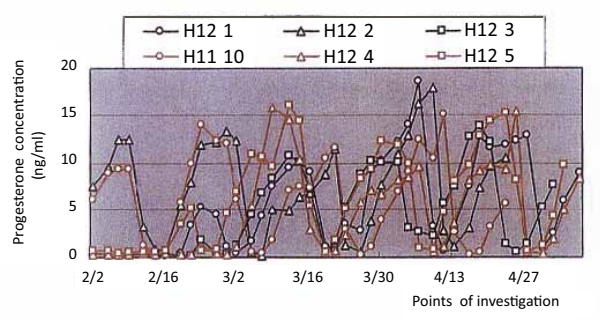

Fig. 22. Changes in plasma progesterone observed in somatic cell cloned cattle (H12-1, H12-2 and H12-3) and conventionally bred cattle (H11-10, H12-4 and H12-5)
(Females, Holstein dairy cattle, cited from reference #38 with permission)

Studies using reproductive technologies such as AI and *in vitro* fertilization (IVF) demonstrated that bulls derived from somatic cell cloning technology could use as sires like their nuclear donors (Table 5, 6). Similarities in calf production rate in AI and IVF were also observed among somatic cell cloned bulls, their nuclear donor and conventionally bred bulls[58,64] (Table 6). With regard to somatic cell cloned cows, most of them showed normal estrus cycles after they reached puberty (Table 7). No abnormalities in their plasma progesterone concentrations were found (Figure 22). Their reproductive performances were confirmed by investigations using reproductive technologies such as AI (Table 8), multiple ovulation and ET (Table 9); however, one cow was identified

Table 7. Occurrence of estrus and ovulation observed in somatic cell cloned cows and conventionally bred cows

Kind of cows	Cow #	Date of estrus observed	Date of ovulation observed	Estrous cycle (days)	Note	Kind of cows	Cow #	Date of estrus observed	Date of ovulation observed	Estrous cycle (days)	Note
Somatic cell cloned cattle	H12-1	2/17/2001	2/18/2001		The first ovulation	Conventionally breed cattle	H11-10	2/13/2001	2/14/2001		
	Born on	3/3/2001	3/4/2001	14			12/16/1999	3/5/2001	3/6/2001	20	
	2/19/2000	3/20/2001	3/21/2001	17				3/25/2001	3/26/2001	20	
		4/7/2001	4/8/2001	18				4/15/2001	4/16/2001	21	
		4/27/2001	4/28/2001	20			H12-4	-	2/20/2001		The first ovulation
	H12-2	2/14/2001	2/15/2001				3/18/2000	2/26/2001	2/20/2001	7	
	Born on	3/6/2001	3/7/2001	20				3/19/2001	3/20/2001	21	
	3/2/2000	3/24/2001	3/25/2001	18				4/9/2001	4/10/2001	21	
		4/12/2001	4/13/2001	19				4/28/2001	4/29/2001	19	
	H12-3	2/20/2001	2/20/2001		The first ovulation		H12-5	-	2/15/2001		The first ovulation
	Born on	-	2/27/2001	7			4/2/2000	2/25/2001	2/26/2001	11	
	3/2/2000	3/18/2001	3/19/2001	20				3/18/2001	3/19/2001	21	
		4/6/2001	4/7/2001	19				4/7/2001	4/8/2001	20	
		4/24/2001	4/25/2001	18				4/27/2001	4/28/2001	20	
			Average	18 8					Average	20 3	

Note 1: No description of estrus cycle at the first ovulation
Note 2:"-" means no symptom of estrus

(Holstein dairy cattle, cited from reference #38 with permission)

Table 8. Artificial inseminations (AIs) performed in somatic cell cloned cows with semen produced by conventionally bred bulls

Kind of cows	Cow #	Birth day	The first day of AIs	The last day of AIs	Age at conception (Months)	Number of AI treatments	Number of frozen semen straws used for AI treatments
Somatic cell cloned cattle	H12-1	2/19/2000	4/25/2001	11/22/2001	21 1	5	7
	H12-2	3/2/2000	4/14/2001	6/6/2001	15 2	2	2
	H12-3	3/2/2000	4/25/2001	6/2/2001	15 0	2	2
				Average	17 1	3 0	3 7
Conventionally bred cattle	H11-10	12/16/1999	4/15/2001	4/16/2001	16 0	1	2
	H12-4	3/18/2000	5/13/2001	6/4/2001	14 6	2	2
	H12-5	4/2/2000	4/27/2001	4/27/2001	12 8	1	1
				Average	14 5	1 3	1 7

Note: Two AIs in single estrus were counted as one "AI treatment"

(Holstein dairy cattle, cited from reference #38 with permission)

Table 9. Calf production with embryo transfers (ETs) with embryos derived from somatic cell cloned cows

Date of ET	Recipient cows (cow#)	Date of embryo collection from cloned cows	Status of embryos for ETs	Result of ETs
12/17/2003	Cross breed (G32)	12/4/2003	Frozen	Pregnant
12/24/2003	Cross breed (G60)	12/4/2003	Frozen	Pregnant
12/26/2003	Cross breed (G41)	12/4/2003	Frozen	Pregnant
6/10/2004	Cross breed (G24)	6/10/2004	Flesh	Pregnant

Note: The semen used for this experiment was produced by a somatic cell cloned bull

(Japanese Black beef cattle, cited from reference #35 with permission)

as sterile due to calcinosis and artery anomaly of the uterus [26]. Investigations of pregnant cloned cows showed that gestation period, birth weight and perinatal loss of newborns were within the ranges of the breed characteristics and variance of individuals; however, there was one case of stillbirth due to immunodeficiency of the newborn, which was progeny of a cloned cow [73].

With regard to reproductive function of five progeny of cloned cows, five cows were investigated in the UATAFF project #1602. These cows were inseminated artificially by semen produced by conventionally bred bull. As a result, no significant differences were found in birth weight and gestation period when they were compared with those in conventionally bred cattle. After the parturition, similarities in indices including occurrence of the first ovulation and first estrus, plasma progesterone (P4) at the first estrus and number of AIs for first conception between the progeny and conventionally bred cows were found. Moreover, no significant differences in maximum diameter of dominant follicle in estrus phase and maximum diameter of corpus luteum in luteral phase were also observed between these animal groups (NLBC, unpublished data).

3.5 Milk/meat productive performances

3.5.1 Milk productive performance

Twenty-two somatic cell cloned cattle were employed for investigation of milk productive performance [4,16,20,22-25,40,68,73,77,80] (Table 2). These clones were at Developmental Node 5 (post-puberty and aging). The findings concerning milk productive performance including milk yield, lactation curves and three to 11 milk quality parameters including total fat, total protein, somatic cell counts, non-urea nitrogen lactose and solids-not-fat (SNF) obtained from somatic cell cloned cows varied within the ranges of breed properties and individual differences (Figures 10-1 and 10-2). Although some parameters such as

Table 10-1. Quality of milk produced by a somatic cell cloned cow and its nuclear donor on the second lactation

		Investigation			
		1st	2nd	3rd	Mean±SD
Milk fat (%)	Nuclear donor	4 69	4 12	3 33	4 05±0 68
	Somatic cell cloned cow	4 55	3 30	2 02	3 29±1 27
Solids-not-fat (%)	Nuclear donor	8 99	9 04	7 85	8 63±0 67
	Somatic cell cloned cow	9 11	8 84	9 02	8 99±0 14
Milk protein (%)	Nuclear donor	4 09	4 2	3 47	3 92±0 39
	Somatic cell cloned cow	3 48	3 47	3 58	3 51±0 06
Lactose (%)	Nuclear donor	3 90	3 84	3 38	3 71±0 28[a]
	Somatic cell cloned cow	4 63	4 37	4 44	4 48±0 13[b]
Somatic cell counts	Nuclear donor	247	239	147	211 0±55 57
	Somatic cell cloned cow	88	230	159	159 0±71 00
MUM (mg/100ml)	Nuclear donor	7 42	9 66	4 05	7 04±2 82[c]
	Somatic cell cloned cow	10 94	11 59	8 26	10 26±1 77[d]

a,b: Within each groups, rows with different superscripts differ $P<0\ 05$
c,d: Within each groups, rows with different superscripts differ $P<0\ 05$

(Holstein dairy cattle, cited from reference #80 with permission)

Table 10-2. Quality of milk produced by a somatic cell cloned cow and its nuclear donor on the second lactation (continued)

		Investigation			
		1st	2nd	3rd	Mean±SD
Casein (g/100ml)	Nuclear donor	2.5	2.8	2.5	2.60±0.17
	Somatic cell cloned cow	2.5	2.8	2.6	2.63±0.15
Saturated fatty acids (g/100ml)	Nuclear donor	2.25	2.41	2.65	2.44±0.20
	Somatic cell cloned cow	2.48	2.89	2 39	2.59±0.27
Non-saturated fatty acids (g/100ml)	Nuclear donor	1.89	1.75	1.45	1.70±0.22[a]
	Somatic cell cloned cow	0.86	1.17	0.79	0 94±0.20[b]
Ca (g/100ml)	Nuclear donor	119	123	122	121 33±2.08
	Somatic cell cloned cow	123	119	128	123 33±4.51

a,b: Within each groups, rows with different superscripts differ. $P<0.05$.

(Holstein dairy cattle, cited from reference #80 with permission)

Table 11. Milking record of somatic cell cloned cows and conventionally bred cows in the first lactation

	Milk yield (kg/day)	Estimated lactation yield (kg)	Milk fat (%)	Milk protein (%)	Solids-not-fat (%)	Body weight (kg)
Nuclear donor	28.8	10,357	3.2	3.3	9.1	567.1
H12-1	30.9	12,005	4.2	3.5	9.0	784.6
H12-2	27.8	9,433	3.9	3.5	9.2	645.1
H12-3	20.8	6,183	4.2	3.5	9.2	737.9
Clones	26.9	10,598	4.1	3.5	9.2	740.9
*Clones	29.3	10,719	4.1	3.5	9.1	714.9
H12-6	27.6	10,259	4.3	3.8	9.4	678.1
H12-7	32.4	12,055	4.0	3.4	8.9	592.1
H12-8	25.6	9,819	3.9	3.2	8.8	548.4
AI cattle	28.1	10,711	4.2	3.5	9.1	615.8

Note 1: H12-1, H12-2 and H12-3 are somatic cell cloned cattle.
Note 2: "Clones" means average of three somatic cell clones.
Note 3: H12-6, H12-7 and H12-8 are conventionally bred cattle by artificial insemination (AI).
Note 4: "AI cattle" means average of conventionally bred cattle.
*Clones: Average expecting H12-3, since its investigation was gave up due to accident during the lactation period.

(Holstein dairy cattle, cited from reference #40 with permission)

lactose showed significant difference between clones and conventionally bred cows, the differences seemed to be slight. With regard to milk yield, a relatively large variance was observed between the cloned cows and their nuclear donors, although these cows had the same genetic backgrounds (Table 11). The appearance of such variations might be due to differences in feeding conditions [77].

Milk productive performances in five progeny produced by cloned cows derived from a same nuclear donor were compared with five conventionally bred cows as a part of the UATAFF project #1602. This would be the only investigation concerning milk productive performance in progeny of clones carried out in Japan. Milk yield of the progeny and conventionally bred cows in a period of 305 days was 10,154 and 12,584 kg respectively. The milk quality parameters including total fat, total protein, lactose and SNF showed higher values in progeny of clones, however, these were not abnormal ranges. It should be noted that that relatively small variances were observed in milk yield and total fat (NLBC, unpublished data).

3.5.2 Meat productive performance

Nineteen somatic cell cloned cattle were employed for investigation of meat productive performance [5,8,35,50,51,57,71] (Table 2). The core observations were carried out using clones/progeny at the Developmental Node 5 (post-puberty maturation and aging) except

for the records concerning body weight gain, which were obtained from clones/progeny not only at Developmental Node 5 but also at much earlier Developmental Nodes. The fattening trials provided meat production data, such as body weight gain [71] (Figure 23 and Table 12), carcass traits [71] (Table 13) and physicochemical properties of hysicochemical properties [71] (Table 14). Similarities in body weight gain and carcass traits were observed between the nuclear donors and clones originating from the same nuclear donor. The values obtained for the clones from these observations were within the normal ranges.

With regard to progeny of somatic cell cloned cattle, 23 cattle were employed for investigations of meat productive performances [47,49,56,60] (Table 3). The fattening trials provided meat production data, such as body weight gain [56] (Figure 24), carcass traits [56] (Table 15), amino acids [48] (Table 16) and fatty acids [47] (Table 17). Although some variations in parameters of meat productive performances observed in the progeny due to health status, the values were within the normal rages [56] (Figure 24). It seems that the same fattening results could be expected from progeny and their half siblings [56] (Table 15).

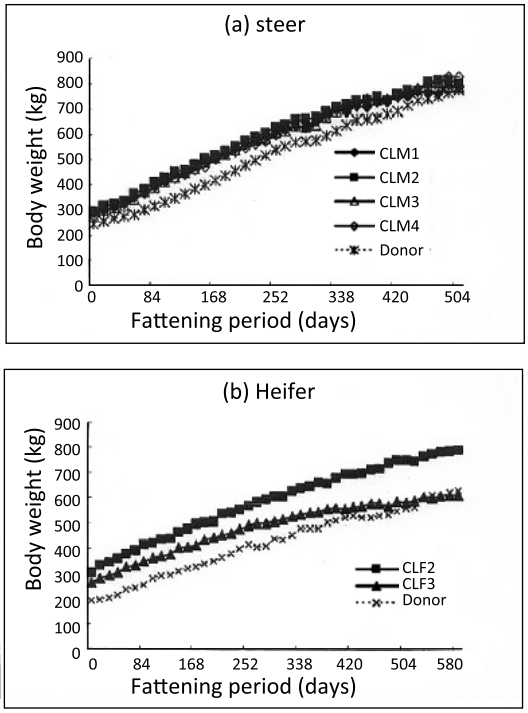

Fig. 23. Increase of body weight during fattening trials observed in somatic cell cloned cattle and their nuclear donors
(Japanese Black beef cattle, cited from reference #71 with permission)

Table 12. Daily gain of somatic cell cloned cattle and its nuclear donor during fattening trials

	Group	Daily gain (kg/day)			
		in the first period[2]	in the middle period[2]	in the latter period[3]	total
Steer	Donor	1.03	1.17	0.93	1.04
	Clone				
	CLM1	1.30	1.13	0.49	0.97
	CLM2	1.32	1.13	0.56	1.00
	CLM3	1.35	1.11	0.55	1.00
	CLM4	1.34	1.13	0.79	1.09
	Average of clone	1.33	1.13	0.60	1.02
	SD of clone	0.02	0.01	0.13	0.05
	CV of clone	1.8	1.1	22.0	4.8
	Max of clone	1.35	1.13	0.79	1.09
	Min of clone	1.30	1.11	0.49	0.97
	Difference[1]	0.05	0.02	0.30	0.11
Heifer	Donor	0.81	0.89	0.59	0.74
	Clone				
	CLF1	1.14	0.83	0.61	0.83
	CLF2	0.92	0.69	0.31	0.59
	Average of clone	1.03	0.76	0.46	0.71
	Difference[1]	0.22	0.14	0.30	0.23

CV: coefficient of variation

[1] Difference means difference between maximum and the minimum value in the steer and the difference of two clones of the heifer.

[2] Both of the first period and middle period were for 168 days

[3] The latter periods were for 168 days in steer and 252 days in heifer

(Japanese Black beef cattle, cited from reference #71 with permission)

Table 13. Carcass traits of somatic cell cloned cattle and its nuclear donor after fattening trials

	Group	Carcass weight (kg)	Grade	Rib eye area (cm^2)	Grade	Rib thickness (cm)	Subcutaneous fat thickness (cm)
Steer	Donor	473	A-4	48	5	8.3	1.8
	Clone				7		
	CLM1	486	A-4	51	8	8.1	1.6
	CLM2	499	A-5	54	7	8.9	1.9
	CLM3	483	A-4	48	9	8.3	2.2
	CLM4	512	A-5	55		8.8	1.6
	Average of clone	495.0		52.0	7.8	8.5	1.8
	SD of clone	13.3		3.2	1.0	0.4	0.3
	CV of clone	2.7		6.1	12.4	4.5	15.7
	Max of clone	512		55	9	8.9	2.2
	Min of clone	483		48	7	8.1	1.6
	Difference[1]	29		7	2	0.8	0.6
Heifer	Donor	377	A-5	59	9	7.0	2.0
	Clone						
	CLF1	489	A-5	74	10	9.2	1.0
	CLF2	365	A-4	53	8	7.0	2.6
	Average of clone	427		63.5	9.0	8.1	1.6
	Difference[1]	124		21	2	2.2	1.6

CV: coefficient of variation.

[1] Difference means difference between maximum and the minimum value in the steer and the difference of two clones of the heifer

(Japanese Black beef cattle, cited from reference #71 with permission)

Table 14. Physiological properties of *M. longissimus thoracis* derived from somatic cell cloned cattle and its nuclear donor after fattening trials

	Group	Moisture (%)	Ether extract (%)	Crude protein (%)	Cooking loss (%)	Shear force value (lb/cm^2)	Water holding capacity (%)
Steer	Donor	50.98	32.87	15.63	-	-	-
	Clone						
	CLM1	52.78	31.05	15.67	24.71	4.07	80.28
	CLM2	48.66	37.15	13.99	22.46	3.37	82.62
	CLM3	51.02	33.94	14.59	25.22	3.80	81.89
	CLM4	46.26	39.60	13.99	21.25	3.94	85.19
	Average of clone	49.68	35.43	14.56	23.41	3.79	82.50
	SD of clone	2.84	3.73	0.79	1.87	0.31	2.04
	CV of clone	5.7	10.5	5.5	8.0	8.0	2.5
	Max of clone	52.78	39.60	15.67	25.22	4.07	85.19
	Min of clone	46.26	31.05	13.99	21.25	3.37	80.28
	Difference[1]	6.52	8.56	1.68	3.97	0.7	4.91
Heifer	Donor	51.04	32.94	15.64	-	-	-
	Clone						
	CLF1	50.05	34.21	15.26	23.72	4.82	79.22
	CLF2	49.87	34.53	15.08	23.67	4.30	80.50
	Average of clone	49.96	34.37	15.17	23.70	4.56	79.86
	Difference[1]	0.18	0.32	0.18	0.05	0.52	1.28

CV: coefficient of variation.

[1] Difference means difference between maximum and the minimum value in the steer and the difference of two clones of the heifer.

(Japanese Black beef cattle, cited from reference #71 with permission)

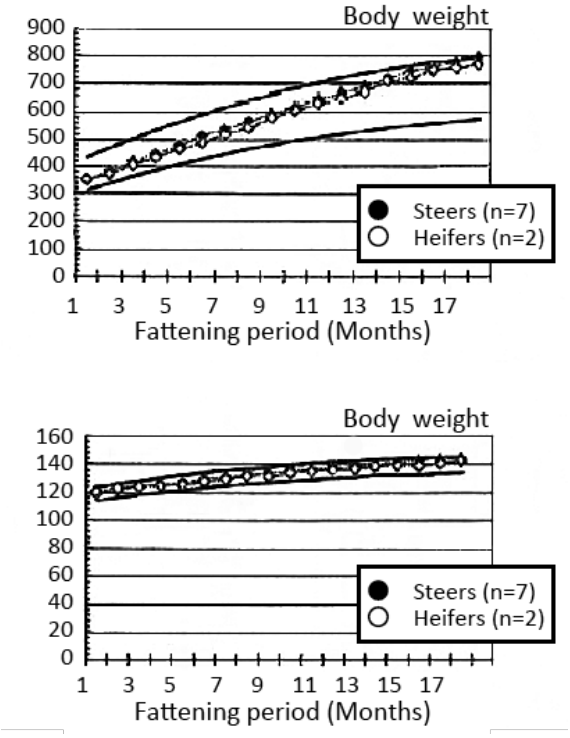

Fig. 24. Increase of body weight during fattening trials observed in progeny of clones (Japanese Black beef cattle, cited from reference #56 with permission)

Table 16. Amino acid composition of *M. longissimus thoracis* derived from progeny of somatic cell cloned cattle and conventionally bred cattle

(g/100g)

	Progeny of clones [1]	Conventionally bred cattle [2]
Arginine	0.97±0.08	0.95±0.11
Lysine	1.35±0.11	1.32±0.17
Histidine	0.59±0.06	0.59±0.08
Phenylalanine	0.59±0.05	0.57±0.07
Tyrosine	0.53±0.05	0.51±0.06
Leucine	1.22±0.10	1.20±0.15
Isoleucine	0.69±0.06	0.68±0.08
Methionine	0.41±0.03	0.40±0.05
Valine	0.72±0.06	0.71±0.08
Alanine	0.85±0.07	0.83±0.10
Glycine	0.62±0.05	0.63±0.07
Proline	0.58±0.04	0.57±0.07
Glutamic acid	2.29±0.19	2.23±0.28
Serine	0.57±0.05	0.55±0.06
Threonine	0.69±0.06	0.67±0.08
Aspartic acid	1.40±0.11	1.37±0.17
Tryptophan	0.19±0.02	0.18±0.03
Cystine	0.18±0.02	0.17±0.02

Mean±SD

[1] n=4

[2] n=6

(Japanese Black beef cattle, cited from reference #48 with permission)

Table 15. Carcass traits of progeny derived from a somatic cell cloned bull (YUMEFUKU) and its nuclear donor (ITOFUKU) after fattening trials

	Sire used for production of steers		Sire used for production of heifers	
Items	ITOFUKU (n=1,410)*	YUMEFUKU (n=7)	ITOFUKU (n=142)*	YUMEFUKU (n=2)
Carcass weight (kg)	443.94±44.05	515.7±30.31	489.02±43.22	488.4
Rib eye area (cm^2)	51.99±7.19	54.3±6.69	48.64±6.64	57.5
Rib thickness (cm)	7.12±1.03	9.1±0.82	6.64±1.16	8.0
Subcutaneous fat thickness (cm)	2.97±1.03	4.5±0.64	3.20±1.05	4.9
BMS#	6.85±2.24	7.0±2.27	5.67±2.12	7.0
Daily gain (kg/day)	0.76±0.13	0.89±0.07	0.66±0.14	0.86

*) Among progeny of ITOFUKU, which produced 6,563 steers and 613 heifers, animals sent to market at 870-900 days old were shown.

Note) ITOFUKU: nuclear donor, YUMRFUKU: somatic cell cloned cattle derived from ITOFUKU.

(Japanese Black beef cattle, cited from reference #56 with permission)

Table 17. Fatty acid composition of fat in muscle derived from progeny of somatic cell cloned cattle and conventionally bred cattle

(%)

	Progeny of clones[1]	Conventionally bred cattle[2]
Lauric acid (C12:0)	0.0±0.0	0.0±0.0
Myristic acid (C14:0)	2.2±0.4	2.2±0.3
Palmitic acid (C16:0)	22.7±2.1	22.8±1.4
Palmitoleic acid (C16:1)	4.8±0.6	4.3±0.4
Stearic acid (C18:0)	9.5±0.6	10.4±0.8
Oleic acid (C18:1)	58.5±2.2	52.8±1.9
Linoleic acid (C18:2)	2.2±0.2	2.1±0.5
Linolenic acid (C18:3)	0.1±0.0	0.1±0.1
Saturated fatty acids	34.4±2.5	35.4±2.2
Mono-unsaturated fatty acids	63.3±2.5	62.5±1.8
Poly-unsaturated fatty acids	2.3±0.2	2.2±0.6
Unsaturated fatty acids	65.6±2.5	64.6±2.2

Mean±SD
[1] n=4
[2] n=8
[3] Saturated fatty acids = C12:0 + C14:0 + C16:0 + C18:0
[4] Mono-unsaturated = C16:1 + C18:1
[5] Poly-unsaturated fatty acids = C18:2 + C18:3
[6] Unsaturated fatty acids = C16:1 + C18:1 + C18:2 + C18:3
(Japanese Black beef cattle, cited from reference #47 with permission)

4. Findings concerning characteristics of animal products derived from somatic cell cloned cattle and their progeny obtained in Japan

The composition analyses of animal products derived from somatic cell cloned cattle might be designated as the only data for characteristics of animal products on food safety assessment in western countries. In the case of Japan, wide-ranging data concerning characteristics of animal products would be required for food safety assessment of food products derived from clones due to its nervous national characteristic.

The characteristics of novel foods such as animal products derived from somatic cell cloned cattle and their progeny should be investigated by an approach based on practical equivalence. The approach would be effective for investigating a possibility that new types of unknown proteins, which might be produced by animal cloning procedure, behave as allergens and/or carcinogens in laboratory animals that fed animal product derived from somatic cell cloned cattle and their progeny.

The approach was put into practice by two investigations, "Investigation on the attributes of cloned bovine products" [14] and "Characteristics of milk/meat derived from progeny of somatic cell cloned cattle" [43]. The latter investigation, which performed as a part of the UATAFF project #1602, was "progeny version" of the former investigation. Therefore, they were compensating for each other on findings concerning characteristics of animal products derived from somatic cell cloned cattle and their progeny.

4.1 Employed cattle for milk/meat production

For the investigations of animal products derived from clones, tree of each embryonic cell cloned cows, somatic cell cloned cows and conventionally bred cows were employed for producing milk [14]. And one embryonic cell cloned steer, one somatic cell cloned steer and three conventionally bred steers were fattened up for meat production [14]. For investigations of animal products derived from progeny, tree of each progeny of somatic cell cloned cows and conventionally bred cows were employed for producing milk [43]. And three of each progeny (heifer) of somatic cell cloned cattle and conventionally bred heifers were fattened up for meat production [43]. Breed of cattle employed for milk/meat production were Holstein and Japanese Black (Wagyu), respectively.

4.2 Hematology and clinical chemistry on cattle employed for milk/meat production

Blood was collected from the jugular vein for analyses concerning hematology and clinical biochemistry. The blood collections were carried out from cloned cows and conventionally bred cows at 3, 6, and 9 months of pregnancy and 3 and 6 weeks after birth in the case of dairy cattle and 3 to 4 times during a period from 21 to 28 weeks after birth in the case of beef cattle [14,43].

The blood collected from cattle was analyzed as shown below: RBC, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte count, WBC,

differential leucocyte count and platelet count. The following clinical chemistry tests were also performed: LDH, aspartate aminotransferase/glutamic-oxaloacetic transaminase (AST/GOT), alanine aminotransferase/glutamic-pyruvic transaminase (ALT/GPT), creatine kinase (CK), ALP, γ–GTP, cholinesterase (ChE), total protein, albumin, globulin, albumin/globulin (A/G) ratio, total cholesterol, triglyceride, phospholipid, glucose, total bilirubin, BUN, creatinine, calcium, inorganic phosphorous, Na,

Table 18. Range of hematology and clinical chemistry parameters investigated in progeny of clones and reference data

Data source	Number of heifers investigated	RBC (10^4/μl)	Hemglobin (g/dl)	Hematocrit (%)	MCV (fl)	MCH (pg)	MCHC (%)	Platelet (10^4/μl)	PT (sec)
Progeny of cloned cattle*	4	701 - 806	12.5 - 15.4	36.0 - 42.5	48 - 55	16.5 - 19.1	34.2 - 36.1	36 - 52	13.6 - 14.8
Reference data**	35	543 - 971	10.5 - 15.6	31.5 - 44.8	41 - 60	14.1 - 20.7	31.3 - 37.1	15 - 40	12.6 - 14.7

Data source	Number of heifers investigated	APTT (sec)	WBC (10^2/μl)	Differential leukocyte counts (%)					
				Basophil	Eosinophil	Neutrophil		Lymphocyte	Monocyte
						Band	Segmented		
Progeny of cloned cattle*	4	66.9 - 69.6	65 - 140	0 - 1	4 - 10	0 - 4	26 - 55	32 - 62	2 - 3
Reference data**	35	33.1 - 107.5	50 - 112	0 - 1	0 - 16	0 - 2	19 - 63	30 - 69	0 - 7

Data source	Number of heifers investigated	LDH (IU/l)	Isozyme fraction of LDH					AST (IU/l)	ALT (IU/l)
			−1 (%)	−2 (%)	−3 (%)	−4 (%)	−5 (%)		
Progeny of cloned cattle*	4	5070 - 7471	48.6 - 55.1	27.7 - 30.1	12.5 - 16.8	2.3 - 3.7	1.1 - 1.6	61 - 108	15 - 20
Reference data**	36	3042 - 6273	40.1 - 57.7	25.4 - 34.4	11.3 - 20.2	1.4 - 5.5	0.4 - 3.7	23 - 130	15 - 37

Data source	Number of heifers investigated	ALP (IU/l)	γ-GTP (IU/l)	Creatine kinase (IU/l)	Choline esterase (IU/l)	Trigly-ceride (mg/dl)	Total cholesterol (mg/dl)	Phospho-lipid (mg/dl)	Total protein (g/dl)
Progeny of cloned cattle*	4	160 - 269	45 - 91	119 - 154	27 - 32	14 - 23	83 - 126	91 - 133	6.55 - 7.38
Reference data**	36	48 - 283	0 - 101	0 - 791	27 - 51	9 - 34	56 - 205	68 - 216	6.56 - 7.85

Data source	Number of heifers investigated	Albumin (%)	α-Globulin (%)	β-Globulin (%)	γ-Globulin (%)	A/G ratio	BUN (mg/dl)	Uric acid (mg/dl)	Glucose (mg/dl)
Progeny of cloned cattle*	4	40.1 - 49.1	14.1 - 14.7	11.6 - 13.8	24.5 - 31.4	0.67 - 0.97	16.5 - 18.2	0.57 - 0.67	67 - 70
Reference data**	36	35.5 - 48.9	11.2 - 18.5	10.6 - 14.7	23.6 - 37.1	0.54 - 0.94	10.5 - 24.9	0.26 - 1.07	52 - 78

Data source	Number of heifers investigated	Creatinine (mg/dl)	Total bilirubin (mg/dl)	Calcium (mg/dl)	Inorganic phospharus (mg/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)
Progeny of cloned cattle*	4	1.51 - 1.69	0.28	9.0 - 9.2	6.6 - 7.1	145 - 147	4.53 - 4.66	102 - 105
Reference data**	36	1.22 - 1.93	0.18 - 0.32	8.2 - 9.7	5.6 - 7.8	144 - 150	3.90 - 5.10	100 - 106

*: Reared at Oita Prefectural Agriculture, Forestry and Fisheries Research Center. Rage is shown as upper and lower values obtained in the present investigation.

**: Blood samples for reference data were provided from Fukushima Agricultural Technology Centre (9 samples), Nagasaki Prefectural Livestock Experiment Station (15 samples), and Shiga Prefectural Livestock Technology Promotion Center (12 samples). Range is shown as upper (mean+2SD) and lower (mean-2SD) values obtained in the present investigation.

K and Cl.

These investigations showed that embryonic/somatic cell cloned cattle employed for the milk/meat production did not have any health problems. There were also no significant differences in parameters investigated between somatic cell cloned cattle and conventionally bred cattle and cloned cattle employed for the present investigation [14]. With regard to progeny of clones, no remarkable abnormalities suggesting poor health status of the progeny were also observed in the blood parameters investigated in the same manner as did in somatic cell cloned cattle [43] (Table 18).

4.3 Nutritional components of milk/meat

Milk was collected in 3rd and 6th week, and frozen for storage. After thawing, it was mixed in a ratio of morning to night lactation, and used as sample for analysis. With regard to meat, 500 g of each retail cuts including *loin*, *shoulder* and *round* were minced and mixed uniformly and used for analyses.

The general components (water content, protein, lipid, carbohydrate, ash content, calcium (only in milk) and cholesterol), amino acids (essential amino acids [isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan and valine] and non-essential amino acids [histidine, arginine, alanine, aspartic acid, glutamic acid, glycine, proline and serine]) and fatty acids (essential fatty acids [linoleic acid, linolenic acid and arachdonic acid and others], decanoic acid, lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, heptadecenoic acid, stearic acid, oleic acid, arachidic acid, icosenoic acid and icosatrienoic acid) were analyzed with each milk/meat sample. In milk samples, three other fatty acids (butyric acid, hexanoic acid and octanoic acid) were also analyzed.

When the data of milk/meat derived from embryonic/somatic cell cloned cattle were compared with the "Standard Tables of Food Composition in Japan" [36], normal compositions were observed in milk derived from clones and conventionally bred cattle; however higher lipid composition was observed in meat derived from clones and conventionally bred cattle due to high quality of meat [14]. Although there were slight variations in milk/meat compositions were found among individuals, no significant differences in the general components, amino acids, and fatty acid content were evident between milk/meat derived from embryonic/somatic cell cloned cattle and conventionally bred cattle [14,59].

With regard to progeny of clones, higher lipid and lower carbohydrate compositions were observed in milk derived from progeny and conventionally bred cattle, and higher lipid composition was observed in meat derived from progeny and conventionally bred cattle [43], when the analyzed results were compared with the "Standard Tables of Food Composition in Japan" [36]. These findings suggest that the progeny and conventionally bred cattle employed for this study produced high-quality milk/meat. Although individual differences were observed in some indices, no differences in milk/meat compositions due to the origin of milk/meat were observed [43].

4.4 Detection of anaphylactic reaction in milk/meat samples by mouse abdominal wall method

Five-week-old male mice [ddy] reared under specific pathogen-free conditions were used for this study. Each test group consisting of 10 mice was housed in a polycarbonate cage with wood chip bedding and fed commercial pellets with water provided *ad libitum*. They were kept at a settled condition. Three mouse groups were assigned for detection of an anaphylactic reaction for milk/meat samples with elicitation treatment. Another three mouse groups were also assigned for this detection without elicitation treatment. The contents of samples for the detection were milk/meat, and a positive control substance (obalbumin); therefore, six mouse groups were required for the detection of milk/meat samples. Sensitization was carried out by injecting 50 µl of sensitization solution intraperitoneally. Elicitation was performed after 14 days of sensitization. After 1% Evan's blue solution (100 µl/mouse) was injected through the tail vein, the abdominal wall was exposed under ether anesthesia. Five minutes after

injection, elicitation solution (50 µl/site) was injected into the abdominal wall. The length and breadth of the dye leakage (forming a circular shape) on the abdominal wall was then measured 7 minutes after the abdominal wall injection.

The present investigation revealed that there were significant differences in the size of pigment leakage with milk/meat derived from embryonic/ somatic cell cloned cattle and conventionally bred cattle compared with that of negative controls; the results demonstrated the presence of allergens in the milk/meat evaluated here. However, no significant differences in anaphylactic reactions were observed due to the origin of milk/meat powder. Therefore, it can be concluded that the anaphylactic reaction from milk/meat derived from embryonic/somatic cell cloned cattle is equivalent to that derived from conventionally bred cattle [14]. With regard to progeny of clones, no significant differences in anaphylactic reactions due to the origin of milk/meat were also found by injecting the test dosage intraperitoneally to mice [43].

4.5 Digestion test based on protein digestion rate in milk/meat with rats

In accordance with the results of composition analyses of milk/meat powder, the diet was supplemented with each pooled powder to the levels equivalent to the basal diet, AIN93M-purified diet for rodents, with protein concentration of 13.03%. Before the meat powder was added to the diet for the digestion test, fat in meat powder was removed by processing in hot water for 10 minutes, since it had high lipid content. Thirty-nine-week-old male SD rats [Crl:CD (SD)] weighing around 630 g, which could eat the prescribed amount of basal diet (AIN93M) within seven days of pre-feeding test, reared under specific pathogen-free condition, were used for this study. Each test group consisting of five rats was fed one of the four test diets as shown above for eight days with water provided *ad libitum*. The rats were selected by stratified random sampling with their body weight. Each rat was kept in an individual stainless steel cage in an animal room at a settled condition. On the 4^{th} and 7^{th} days of the feeding period, test diets containing 0.1% carmine (red dye) were fed to the rats for 24 hours. The feeding of a carmine-containing diet started at a fixed time. Food consumption was measured for 3 days. This measurement was also started on the 4^{th} day and finished on the 7^{th} day of the feeding period. The feces derived from diets ingested for these 3 days were collected on the basis of the red color of feces due to carmine. The amount of nitrogen in test diet containing milk/meat and the excreted feces was measured by the modified macro-Kieldahl digestion. The digestion rate was calculated with the following formula:

Digestion rate (%) = {[(total nitrogen in the consumed diet)−(total nitrogen in the excreted feces)]/(total nitrogen in the consumed diet)} × 100.

Concerning milk/meat derived from embryonic/ somatic cell cloned cattle and conventionally bred cattle, there were no significant differences in digestibility due to origin of milk/meat [14]. In regard to milk/meat derived from progeny of clones and conventionally bred cattle, no differences in digestion rates were also found due to the origin of milk/meat[43].

4.6 Detection of mutagenicity in milk/meat by mouse micronucleus test

Eight-week-old ICR male mice [Crj:CD-1 (ICR)] reared under specific pathogen-free conditions were used for this study. Each test group consisting of six mice was housed in a polycarbonate cage with wood chip bedding and fed commercial pellets with water provided *ad libitum*. They were kept at a settled condition. Six mouse groups were assigned for detection of mutagenicity in milk powder diets. Each of these groups was fed the test diet for fourteen days as follows: basal diet (for negative control experiment), basal diet (for positive control experiment), diet supplemented with 2 to 10% (w/w) milk powder derived from clones/progeny/conventionally bred cattle and diet supplemented with 1 to 5% (w/w) meat powder derived from clones/progeny/ conventionally bred cattle. For the positive control experiment groups, 2 mg/kg body weight mitomycin C was injected into mice intraperitoneally 24 hours before they were sacrificed. To prepare bone marrow smear, the mice

were sacrificed by dislocation of the cervical vertebrae in the neck after the test diet feeding period. The femur bone was removed immediately and bone marrow cells were washed with fetal bovine serum. The cells were collected by centrifugation at 1,000 rpm for 5 minutes, suspended in saline and smeared on a glass slide. After drying at room temperature, they were fixed with methanol for 5 minutes and stained using Giemsa solution. These slides were observed through a microscope. The frequency of micronucleated cells was determined for one thousand polychromatic erythrocytes per specimen. Significant differences among the negative control group, test sample groups and positive control group were determined. Simultaneously, the ratio of polychromatic erythrocytes to total erythrocytes was also calculated.

In the investigation using milk/meat powder derived from embryonic/somatic cell cloned cattle and conventionally bred cattle, milk powder (maximum 10% (w/w)) and meat powder (maximum 5% (w/w)) in diets were fed to mice for 14 days; however, the rate of multistained red blood corpuscles did not decrease. This result suggests that the substance causing cell toxicity was not contained in the milk/meat powder derived from embryonic/somatic cell cloned cattle as well as conventionally bred cattle [14]. With regard to investigation using milk/meat powder derived from progeny of clones and conventionally bred cattle, it was also concluded that milk/meat derived from these cattle was negative for mutagenicity [43].

4.7 Feeding study in rats

In accordance with the results of composition analyses of milk/meat powder, the diet was supplemented with each pooled powder to the levels equivalent to the basal diet, AIN93G-purified diet for rodents. The percentage of milk/meat supplementation to test diet was decided according to results of a preliminary test (four weeks). Namely, 2 to 10% (w/w) milk powder or 1 to 5% (w/w) meat powder was supplemented to each test diet. These test diets (milk powder diet derived from clones/progeny/conventionally bred cattle and meat powder diet derived from clones/progeny/conventionally bred cattle) were irradiated with γ-rays (10 kGy) and stored at −25℃ until use.

Five-week-old SD rats [Crl:CD(SD)] reared under specific pathogen free condition were used for the feeding study. Each rat group, which consisted of 10 females/males, was fed one of the test diets as shown above for fourteen weeks with water *ad libitum*. They were kept at a settled condition. With regard to feeding study with milk/meat derived from progeny of somatic cell cloned cattle, the feeding period was prolonged to twelve months to observe the effect of test diet on reproductive/performance toxicity in rats. The modification was performed due to suggestion of advice committee of the UATAFF project #1602.

The animals were observed daily for clinical signs; this included examination of outer appearance, behavior, feces and general state. Detailed clinical observations were also carried out monthly. Moreover, grip strength of forelimbs and hindlimbs, motor activity and sensory/reflex function (sound response, approach response, touch response, tail pinch response, pupil reflex to light, pinna reflex, eyelid reflex, ipsilateral flexor reflex and righting reflex) were examined.

To confirm normal growth, the rats were weighed at the beginning of the feeding period (on day 1 of the feeding period), every seven days during the feeding period and the day of sacrifice. The 24-hour food consumption of the rats was also measured once a week. In females, these measurements were suspended during the reproduction/development test. During the feeding period, examinations such as ophthalmology (anterior portion of the eye, chamber, optic media and occular fundus), urinalysis (color, pH, occult blood, protein, glucose, ketone body, bilirubin, urobilinogen, specific gravity and urine volume per 18 hours) were also performed.

At the end of the feeding period, the rats were anesthetized and blood was collected from the abdominal aorta. The following blood tests were preformed using a blood clot automatic measurement device: RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, reticulocyte count, WBC, differential leucocyte count, platelet count; testing for prothrombin time and activated partial thromboplastin time. The

following clinical chemistry tests were performed using an automatic biochemistry analyzer: LDH, AST/GOT, ALT/GPT, CK, ALP, γ–GTP, ChE, total protein, albumin, globulin, A/G ratio, total cholesterol, triglyceride, phospholipid, glucose, total bilirubin, BUN, creatinine, calcium, inorganic phosphorous; testing for Na, K and Cl was also performed.

After the rats were sacrificed for exanguination, necropsy was performed and their organs were weighed. The weights are shown as absolute weights and relative weights (weight/100 g body weight). The organs investigated were as follows: the brain, pituitary gland, thyroid glandlung, heart, salivary glands (sublingual and submandibular), liver, spleen, kidney and adrenal gland for both sexes; testes, epididymis, prostrate and seminal vesicle for males; ovary and uterus for females. Histological examinations of the brain, pituitary gland, eyeball, Harderian gland, thyroid gland, parathyroid, spinal cord, heart, thymus, liver, kidney, spleen, trachea, lung, adrenal gland, salivary gland, tongue, esophagus, stomachmall intestine, large intestine, pancreas, urinary bladder, testis, epididymidis, prostate, seminal vesicle, ovary, uterus, vagina, aorta, sciatic nerve, lymph nodes, bone, bone marrow, skeletal muscle, mammary gland and skin were also conducted.

In rats fed milk/meat derived from progeny of clones, the additional investigations concerning reproduction/development toxicity in rats were carried out as shown bellow. The estrous cycles were examined by vaginal smear for fourteen days comprising the 16th to 17th week of feeding in the female groups fed meat powder diets and the 11th to 12th week of feeding in the

Table 19. Summary of fourteen-week feeding study in rats fed diets supplemented with milk powder derived from embryonic/somatic cell cloned cattle and conventionally bred cattle

		Feeding conditions of rats																	
				Fed diet supplemented with 2 5% (w/w) milk powder derived from;						Fed diet supplemented with 5% (w/w) milk powder derived from;						Fed diet supplemented with 10% (w/w) milk powder derived from;					
		Basal diet		Conven-tionally bred cattle		Embryonic cell cloned cattle		Somatic cell cloned cattle		Conven-tionally bred cattle		Embryonic cell cloned cattle		Somatic cell cloned cattle		Conven-tionally bred cattle		Embryonic cell cloned cattle		Somatic cell cloned cattle	
Items	Sex of rats	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
	Number of rats	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Mortality (shown as number of dead rats)		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Body weight (measured once a week)				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Food consumption (measured once a week)				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sensory/reflex function (9 parameters)				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Grip strength, motor activity (measured at 4 points)				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Estrus cycle					—		—		—		—		—		—		—		—		—
Urinalysis (9 parameters)				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hematology (11 parameters)				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Clinical chemistry (23 parameters)																					
Calcium				▲	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Inorganic phospharus				▽	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
γ-GTP				—	△	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	▲ a)
Organ weight (Male: 14 organs, female: 12 organs)				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pathology				— b)	— b)	— b)	— b)	— b)	— b)	—	— b)	— b)	— b)	— b)	— b)	— b)	— b)	— b)	— b)	— b)	— b)

Note) Significant difference in each item was obtained by comparing between a test group and basal diet group

—: No significant diffrence, △ (P<0 05)・▲ (P<0 01): Significantly increased

▽ (P<0 05)・▼ (P<0 01): Significantly dicreased

a) Not significant difference was found when the data were compared with those of a test group fed diet supplemented with milk derived from conventionally berd cattle

b) Lesions observed in the present investigation were assumed to be spontaneous

female groups fed milk powder diets. The females were mated with the same number of males for up to three weeks. After conceiving, the following items were examined in the dams: estrous cycle, copulation index, fertility index, gestation length and gestation index. During the period from birth until weaning at 21 days, the following items were examined in the pups: litter size, live birth index, sex ratio, body weight, viability index, lactation index, hair growth, pinna detachment, incisor eruption, eyelid opening, testicular descent, sensory response, reflex function tests, external abnormalities and visceral malformations.

In a fourteen-week feeding study of rats with milk/meat derived from embryonic/somatic cell cloned cattle, there were no biologically significant differences in indices of rats concerning health status, development, physiological functions and morphology compared to those in rats fed diet supplemented with milk/meat derived from conventionally bred cattle [14,75] (Tables 19, 20).

In the twelve-month feeding study of rats fed diets supplemented with milk/meat derived from progeny of clones and conventionally bred cattle, there were no biologically significant differences in most of the growth- and reproduction-related induces between the rat groups fed the milk/meat diet derived from the progeny and conventionally bred cattle [43] (Tables 21-1, 21-2, 22-1, 22-2, UATAFF project #1602).

Table 20. Summary of fourteen-week feeding study in rats fed diets supplemented with meat powder derived from embryonic/somatic cell cloned cattle and conventionally bred cattle

		Feeding conditions of rats																			
				Fed diet supplemented with 1% (w/w) meat powder derived from;						Fed diet supplemented with 2 5% (w/w) meat powder derived from;						Fed diet supplemented with 5% (w/w) meat powder derived from;					
		Basal diet		Conven-tionally bred cattle		Embryonic cell cloned cattle		Somatic cell cloned cattle		Conventio nally bred cattle		Embryonic cell cloned cattle		Somatic cell cloned cattle		Conven-tionally bred cattle		Embryonic cell cloned cattle		Somatic cell cloned cattle	
Items	Sex of rats	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
	Number of rats	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Mortality (shown as umber of dead rats)		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Body weight (measured once a week)				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Food consumption (measured once a week)				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sensory/reflex function (9 parameters)				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Grip strength, motor activity (measured at 4 points)				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Estrus cycle					—		—		—		—		—		—		—		—		—
Urinalysis (9 parameters)																					
pH				—	—	—	—	—	△ a)	—	—	—	—	—	▲ b)	—	△	—	—	—	—
Hematology (11 parameters)				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Clinical chemistry (23 parameters)																					
Triglyceride				—	—	—	—	—	—	—	—	—	—	—	—	▲	—	—	—	—	—
BUN				—	—	—	—	—	▼ a)	—	—	—	—	—	△ a)	—	—	—	▽ a)	—	—
Creatinine				—	—	—	—	—	—	—	△	—	—	—	—	—	—	—	—	—	—
Total bilirubin				—	—	—	▽ a)	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Organ weight (Male: 14 organs, female: 12 organs)				—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pathology				— c)	— c)	— c)	— c)	— c)	— c)	—	— c)	— c)	— c)	— c)	— c)	— c)	— c)	— c)	— c)	— c)	— c)

Note) Significant difference in each item was obtained by comparing between a test group and basal diet group

— : Not significant diffrence, △ (P<0 05) ・▲ (P<0 01) : Significantly increased

▽ (P<0 05) ・▼ (P<0 01) : Significantly decreased

a) No significant difference was found when the data were compared with those of a test group fed diet supplemented with meat derived from conventionally bred cattle

b) Significant difference was also found when the data were compared with those of a test group fed diet supplemented with meat derived from conventionally bred cattle

c) Lesions observed in the present investigation were assumed to be spontaneous

4.8 Questionnaires for tasting trials of beef derived from somatic cell cloned cattle and their progeny

To investigate acceptance and understandings of consumers towards somatic cell cloned cattle and their progeny, tasting trials of beef derived from clones/ progeny with questionnaire have been carried out in Japan. Only three reports would be found.

In a questionnaire for tasting trial of beef derived from somatic cell cloned cattle with 161 volunteers who were involved in animal cloning institutions, the volunteers complained "hesitation" for such beef, although most of them answered the beef was "delicious" [5] (Figure 25). In another questionnaire for tasting trial of beef derived from somatic cell cloned cattle with 1,574 volunteers who participate in business circle of domestic animals, 76.3% (100/131, 45 women and 85 men) of them answered that they did not have any "hesitation" to beef derived from clones. Of 31 volunteers who complained "hesitation" to the

Table 21-1. Summary of twelve-month feeding study combined with reproduction/development toxicity test in rats fed diets supplemented with milk powder derived from progeny of somatic cell cloned cattle and conventionally bred cattle (excepting reproduction of dams and observation of their pups)

		Feeding conditions of rats							
		Fed diet supplemented with 2% (w/w) milk powder derived from;				Fed diet supplemented with 10% (w/w) milk powder derived from;			
		Conventionally bred cattle	Progeny of cloned cattle[a)]	Conventionally bred cattle	Progeny of cloned cattle[a)]	Conventionally bred cattle	Progeny of cloned cattle[a)]	Conventionally bred cattle	Progeny of cloned cattle[a)]
Items	Sex of rats	♂		♀		♂		♀	
	Number of rats	12	12	12	12	12	12	12	12
Mortality (shown as number of dead rats)		0	1	0	0	1	0	0	0
Body weight (measured once a week)			—		—		—		—
Food consumption (measured once a week)			—		— b)		—		—
Sensory/reflex function (9 parameters)			—		—		—		—
Grip strength, motor activity (measured at 4 points)			—		—		—		—
Reproductive functions (estrus cycle, copulation index, fertility index, gestation length and gestation index)			—		—		—		—
Ophthalmology			—		—		—		—
Urinalysis (12 parameters)			—		—		—		—
Hematology (12 parameters)									
RBC			—		△ c)		—		—
Hemoglobin			—		△ c)		—		—
Hematocrit			—		△ c)		—		—
WBC			—		—		—		▽ b)
Clinical chemistry (23 parameters)									
Total bilirubin			▲ c)		—		—		—
Inorganic phospharus			—		—		△ b)		—
Total cholesterol			—		▲ c)		—		—
Phospholipid			—		▲ c)		—		—
Organ weight (Male: 14 organs, female: 12 organs)			—		—		—		—
Pathology			— d)		— d)		— d)		— d)

Note) Significant difference in each item was obtained by comparing between test groups fed diet supplemented with milk derived from conventionally bred cattle and progeny of clone

— : Not significant diffrence, △ (P<0 05) · ▲ (P<0 01) : Significantly increased

▽ (P<0 05) · ▼ (P<0 01) : Significantly decreased

a) Progeny of somatic cell cloned cattle

b) Significant differences were observed on 6thand 39th week of the feeding period

c) Within a normal range of reference data

d) Lesions observed in the present investigation were assumed to be spontaneous

Table 21-2. Summary of twelve-month feeding study combined with reproduction/development toxicity test in rats fed diets supplemented with milk powder derived from progeny of somatic cell cloned cattle and conventionally bred cattle (Focused on reproduction of dams and observation of their pups)

Items	Feeding conditions of dams			
	Fed diet supplemented with 2% (w/w) milk powder derived from;		Fed diet supplemented with 10% (w/w) milk powder derived from;	
	Conventionally bred cattle	Progeny of cloned cattle[a)]	Conventionally bred cattle	Progeny of cloned cattle[a)]
Litter size, live birth index, sex ratio, lactation index		—		—
Body weight (measured at 5 points)		— [b)]		—
External abnormalities		—		—
Organ abnormalities		—		—
Visceral malformations (5 parameters)		—		—
Sensory/reflex function (9 parameters)		—		—

Note) Significant difference in each item was obtained by comparing between test groups fed diet supplemented with milk derived from conventionally bred cattle and progeny of clone
－: Not significant difference
a) Progeny of somatic cell cloned cattle
b) Significant differences were observed on the first day of observation

Table 22-1. Summary of twelve-month feeding study combined with reproduction/development toxicity test in rats fed diets supplemented with meat powder derived from progeny of somatic cell cloned cattle and conventionally bred cattle (excepcting reproduction of dams and observation of their pups)

Items	Sex of rats	Feeding conditions of rats							
		Fed diet supplemented with 1% (w/w) meat powder derived from;				Fed diet supplemented with 5% (w/w) meat powder derived from;			
		Conventionally bred cattle	Progeny of cloned cattle[a)]	Conventionally bred cattle	Progeny of cloned cattle[a)]	Conventionally bred cattle	Progeny of cloned cattle[a)]	Conventionally bred cattle	Progeny of cloned cattle[a)]
		♂		♀		♂		♀	
	Number of rats	12	12	12	12	12	12	12	12
Mortality (shown as number of dead rats)		0	1	0	0	1	0	0	0
Body weight (measured once a week)			—		—		—		—
Food consumption (measured once a week)			—		— [b)]		—		—
Sensory/reflex function (9 parameters)			—		—		—		—
Grip strength (measured at 4 points)			— [c)]		—		—		—
Motor activity (measured at 4 points)			— [d)]		—		—		—
Reproductive functions (estrus cycle, copulation index, fertility index, gestation length and gestation index)			—		—		—		—
Ophthalmology			—		—		—		—
Urinalysis (12 parameters)			—		—		—		—
Hematology (12 parameters)									
Monocyte			—		△ [e)]		—		—
Clinical chemistry (23 parameters)									
γ-GTP			—		△ [f)]		—		—
AST			—		—		—		—
BUN			—		—		—		△ [f)]
Inorganic phosphorus									△ [f)]
Na			▲ [f)]		—		▲ [h)]		△ [f)]
Organ weight (Male: 14 organs, female: 12 organs)			—		—		—		—
Liver			△ [h)]		—		—		—
Spleen			—		△ [h)]		—		—
Pathology			— [i)]		— [i)]		— [i)]		— [i)]

Note) Significant difference in each item was obtained by comparing between test groups fed diet supplemented with meat derived from conventionally bred cattle and progeny of clone
－: Not significant diffrence, △ (P<0 05) ・▲ (P<0 01) : Significantly increased
▽ (P<0 05) ・▼ (P<0 01) : Significantly decreased
a) Progeny of somatic cell cloned cattle
b) Significant differences were observed on 37th week of the feeding period
c) Significant differences were observed in hind limb grip on 3rd and 6th week of the feeding period
d) Significant differences were observed on 3rd week of the feeding period
e) Slightly differences were observed
f) Within a normal range of reference data
g) Out of a normal range of reference data slightly
h) The differences were occurred due to corpulence
i) Lesions observed in the present investigation were assumed to be spontaneous

beef, 54.8% (17/31) were women (Tokachi station, NLBC) [46].

With regard of questionnaire for tasting trial of beef derived from progeny of somatic cell cloned cattle with 497 volunteers who were involved in animal cloning institutions, they also complained "hesitation" to such beef, although most of them answered the beef was "delicious" [60] (Figure 26). Another questionnaire for tasting trial of beef derived from progeny of somatic cell clones with 706 volunteers who participated or non-participated in business circle of domestic animals demonstrated interesting result; the percentages of volunteers complained "hesitation" to beef derived from progeny of clones on "participated" and "non-participated" in the business circle were 27.1% (99/364) and 46.5% (33/71), respectively (MIC Co. Ltd., unpublished data).

5. Postscript of the report

Although there are no processes for intentional gene modification in the animal cloning, some "intentional effects" such as epigenetic error of gene have been observed in somatic cell cloned animals [1].

Table 22-2. Summary of twelve-month feeding study combined with reproduction/development toxicity test in rats fed diets supplemented with meat powder derived from progeny of somatic cell cloned cattle and conventionally bred cattle (Focused on reproduction of dams and observation of their pups)

Items	Feeding conditions of dams			
	Fed diet supplemented with 1% (w/w) meat powder derived from;		Fed diet supplemented with 5% (w/w) meat powder derived from;	
	Conventionally bred cattle	Progeny of cloned cattle[a)]	Conventionally bred cattle	Progeny of cloned cattle[a)]
Litter size, live birth index, sex ratio, lactation index		–		–
Body weight (measured at 5 points)		–		–
External abnormalities		–		–
Organ abnormalities		–		–
Visceral malformations (5 parameters)		–		–
Sensory/reflex function (9 parameters)		–		–

Note) Significant difference in each item was obtained by comparing between test groups fed diet supplemented with meat derived from conventionally bred cattle and progeny of clone

– : Not significant difference

a) Progeny of somatic cell cloned cattle

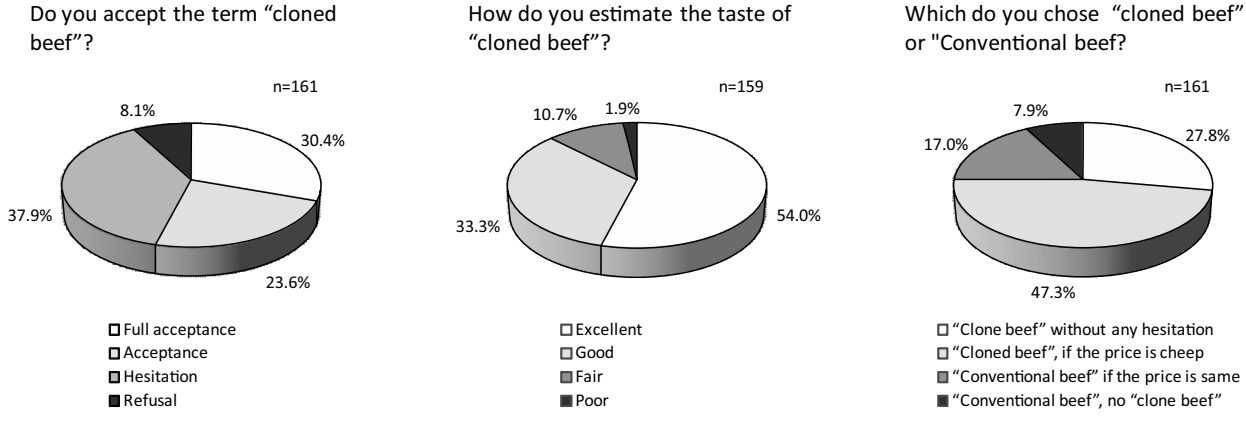

Fig. 25. Results of questionnaire with tasting trail of beef derived from somatic cell cloned cattle, "clone beef" (Japanese Black beef cattle, cited from reference #5 with permission)

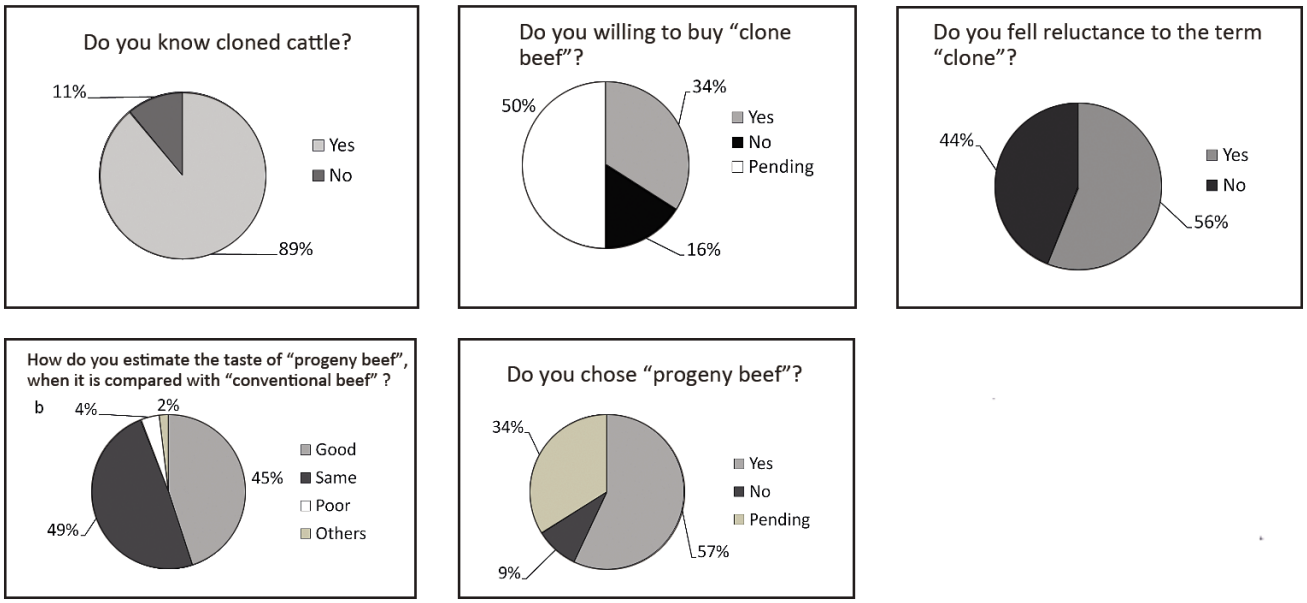

Fig. 26. Results of questionnaire with tasting trail of beef derived from progeny of somatic cell cloned cattle, "progeny beef" (Japanese Brown beef cattle, cited from reference #60 with permission)

Such "intentional effects" might be affect something to food products derived from somatic cell cloned animals and their progeny in occasionally. Therefore, multifaceted data concerning toxicity and nutrition should be obtained for investing about characteristics of food products derived these animals. Such data were obtained by Research Institute for Animal Sciences in Biochemistry & Toxicology, which had authentication including "Good Laboratory Practice" for toxicity and registered inspecting station by the "Food Sanitation Low" of Japan.

Moreover other multifaceted data concerning physiology and clinical veterinary were required for investigating health status of somatic cell cloned animals and their progeny, since it is believed that healthy animals would produce food products with good quality. These data were submitted by Japanese researchers who had been engaged to animal cloning technology for long time. It would be quite difficult to obtain such data without their favor and support. The present report should be dedicated to them.

The author wishes that the present report would be used for risk assessment of food products derived from somatic cell cloned animals and their progeny in the world. The somatic cell cloning technology, which would give many benefits to human society, would make promising feature of animal production. To make it possible, the users of the animal cloning technology should understand multifaceted responsibility including ethics, animal welfare, compliance, risk communication and disclosure of information.

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(Supplement) Somatic Cell Cloned Pigs and Their Progeny Produced in Japan

1. Production and their lifetime

According to the press release by MAFF on October 31, 2007, 256 somatic cell cloned cattle was produced in Japan since July 2, 2000 [2)] (birthday of the first somatic cell cloned cattle produced in Japan). The major breeds of clones were cross-bred, Jin-Hua, Landrace and Duroc. These clones were produced by 6 institutions including 3 prefectural institutions.

2. Production and death/slaughter

To obtain lifetime data including production and death/slaughter of somatic cell cloned pigs (non-genetically modified animals) and their progeny, a survey was carried out in July, 2007. As a result, lifetime data concerning 90 somatic cell cloned pigs and 145 progeny of clones were provided by 3 Japanese institutions. The proportion of cloned pigs obtained their lifetime data estimated only 32.5% (90/256), since most of these cloned pigs were genetically modified animals for medical studies such as creating disease models for human diseases in pigs. Although the number of cloned pigs and their progeny were limited, obtained findings were reviewed here.

Figure A shows production of somatic cell cloned pigs without genetically modification. The major breeds of somatic cell cloned pig and their progeny were cross-bred (81.1%; 73/90) and Jin-Hua (75.2%; 109/145), respectively (Figure B). The kinds of donor cells used for producing cloned pigs were derived from adipose tissue (45.6%; 41/90) and salivary grand (31.1%; 28/90) (Figure C).

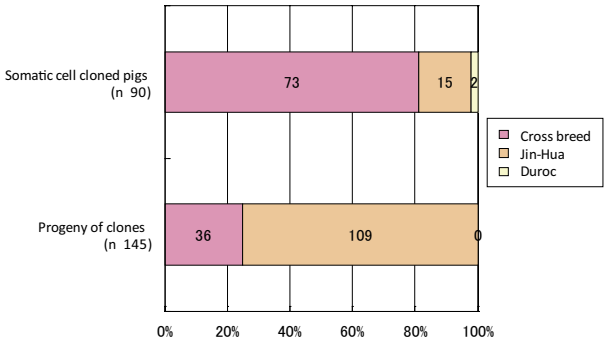

Fig. B. Breeds of somatic cell cloned pigs and their progeny listed in the present survey

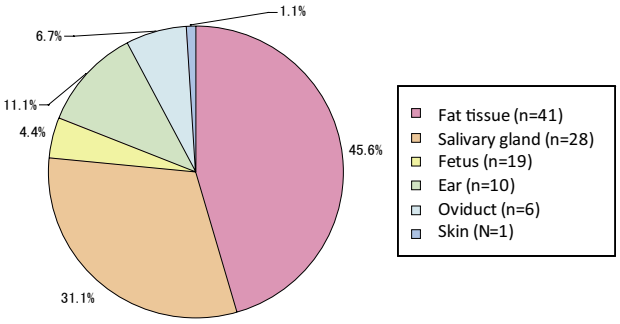

Fig. C. Sources of donor cells used for pig cloning listed in the present survey (n=90)

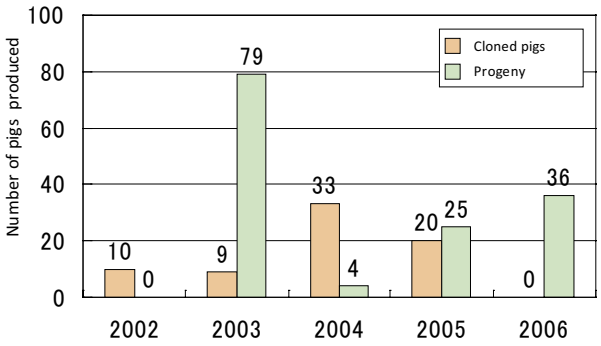

Fig. A. Number of somatic cell cloned pigs and their progeny listed in the present survey

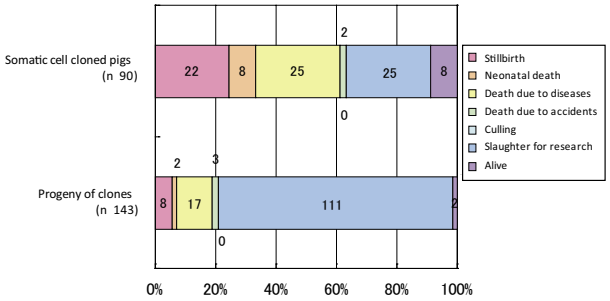

Fig. D. Status of somatic cell cloned Pigs and their progeny at the time of a survey (All pig s surveyed)

Of all non-genetically modified cloned pigs investigated, the incidence of death loss due to stillbirth and neonatal death were 24.4 (22/90) and 8.9% (8/90), respectively (Figure D). When the data were compared with those obtained from somatic cell cloned cattle (stillbirth: 16.4%; 79/482, neonatal death: 15.1%; 73/482), the higher incidence of stillbirth and lower incidence of neonatal death might be feature of somatic cell cloned pig production. It should be noted that 97.8% (88/90) of piglets were produced by induced partition.

In birth weights of somatic cell cloned pigs, almost no large offspring syndrome cases were observed. Namely, the birth weights of cloned piglets (cross-breeds, both sexes) in stillbirth, neonatal death and survived perinatal period were 609.1±377.1 (n=20, mean±SD), 445.2±193.0 (n=6) and 1001.3±342.5g (n=47), respectively. However, in a case of survived Jin-Hua piglets (both sexes), heavier birth weight (915.9±207.4g; n=11) were observed when these were compared those from conventionally bred piglets (763.5±150.3g; n=33). Of the piglets survived the perinatal period, 27.8% (25/90) dead by diseases. The incidence was higher than that of somatic cell cloned cattle (19.5%; 94/482).

With regard of progeny of non-genetically modified somatic cell cloned pigs, incidence of stillbirth, neonatal death were 5.6 (8/143) and 1.4% (2/143), respectively (Figure D). All of these piglets produced by natural parturition with no large offspring syndrome cases. Namely, the birth weights of the progeny (Jin-Hua, both sexes) in stillbirth, neonatal death and survived perinatal period were 562.5±209.7 (n=4), 450 (n=1) and 725.2±139.8g (n=108), respectively. The birth weight obtained from piglets survived perinatal period were similar to that from conventionally bred piglets (763.5±150.3g (n=33). In these cases of parturition, litter size of the progeny and conventionally bred sows were 10.9±3.1 (n=10) and 8.3±1.9 (n=4), respectively. In the piglets survived the perinatal period, 11.9% (17/143) dead by diseases. Most of somatic cell cloned pigs and their progeny survived perinatal period were slaughtered for research (Figure D). The major purpose was sampling of studies.

Table A. Hematology parameters in somatic cell cloned pigs and conventionally bred pigs

	Unit	Mean	Reference rage	Conventionally bred pigs				Somatic cell cloned pigs			
				Mean (n=3)	N_1	N_2	N_3	Mean (n=3)	C_1	C_2	C_3
RBC	(10^4/μl)	683	503～864	538	550	552	512	640	635	680	606
Hemglobin	(g/dl)	13.7	11.2～16.2	12.5	11.6	11.2	14.8	13.6	13.4	13.8	12.1
Hematocrit	(%)	43.2	34.7～51.7	38.6	38.6	39.4	37.9	43 2	44.2	45.3	40.2
MCV	(fL)	64	56～72	71.9	70.2	71.4	74	68.1	69.6	66.6	66.3
MCH	(pg)	20.2	17.2～23.2	20.1	21.1	10.3	28.9	20.5	21.1	20.3	20
MCHC	(%)	31.7	28.9～34.5	32.5	30.1	28.4	39.1	30.4	30.3	30.5	30.1
Reticulocyte	(‰)	8*	0～21.8*	9.7	8.7	10.1	10.2	14.9	12.5	17.3	14.8
Platelet	(10^4/μl)	24	13～35	20.5	-	20.5	-	20 5	18.2	22	21.2
WBC	(10^2/μl)	135	88～182	98.8	84.5	124	87.9	117.7	130.7	108.1	114.2
Basophil	(%)	1.1	0～5	1	2	0	1	0.3	0	0	1
Eosinophil	(%)	2.8	0～7	0.67	0	0	2	0	0	0	0
Band neutrophil	(%)	0.5	0～4	0.67	2	0	0	2.7	4	3	1
Segmented neutrophil	(%)	32	22～49	46.7	45	55	40	62.7	71	56	61
Lymphocyte	(%)	61	41～75	44.7	41	43	50	28.5	20	37	33
Monocyte	(%)	2.7	0～7	6.3	10	2	7	4.3	5	4	4
PT	(sec)	11.6*	8.7～14.6*	10.3	10.3	10.2	10.5	9.8	9.8	10	9.7
APTT	(sec)	26.7*	15.0～41.1*	18.9	17.3	17.7	21.8	19.7	19.1	20.3	17

・Based on available reference data
Note) These data were obtained from sows around 18 months in age.

(LWD pigs, cited from reference #1 with permission)

3. Clinical and pathological investigations

In Blood investigation with three somatic cloned sows (LWD, 18 weeks in age), there were no biological differences in parameters concerning hematology (Table A) and clinical chemistry (Table B) were found when these were compared those in conventionally bred sows [1]. With regard to progeny of clones (Jin-Hua), blood investigations, which were carried out at 45, 90 and 135 days after birth, indicated that there were no biological differences in 12 hematological parameters (15 pigs) and 11 clinical chemistry parameters (10 pigs) when these were compared those in conventionally bred pigs [3].

Three somatic cell cloned pigs, which were consisted of two neonatal death cases and one dead case in 139 days of age, were used for pathological stuies [7]. In two neonatal death cases, deformation in legs and hernia of navel were found in a case and bleedings in brain and abdominal were observed in another case. In other dead case, it was diagnosed as pleural pneumonia and corynebacterium infection. These diseases were well known in conventionally bred pigs.

Growth performance of somatic cell cloned pigs and their progeny were investigated in five Jin-Hua clones [4] (Figure E), three Landrace clones [7] and 40 Jin-Hua progeny [3] (Figure F). No biological differences were observed in growth performance parameters including body weight gain among clones, their

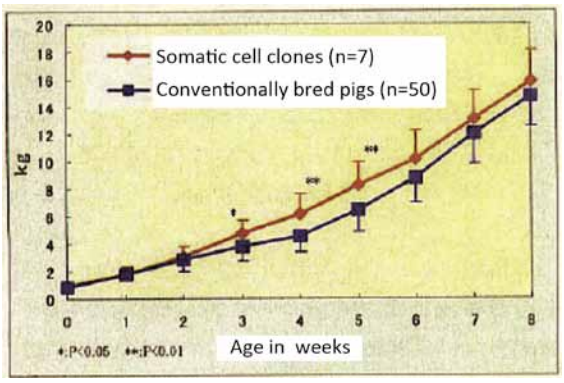

Fig. E. Growth curves observed in a somatic cell cloned pigs and conventionally bred pigs (Females, Jin-Hua Pigs, cited from reference #4 with permission)

Table B. Hematology parameters in somatic cell cloned pigs and conventionally bred pigs

	Unit	Mean	Reference rage	Conventionally bred pigs				Somatic cell cloned pigs			
				Mean (n=3)	N_1	N_2	N_3	Mean (n=3)	C_1	C_2	C_3
LDH	(IU/l)	882	544～1220	931.2	951.8	827.1	1014.7	1182.6	1022.5	1094	1431.4
GOT	(IU/l)	29	15～43	19.8	17.1	21.6	20.8	24.6	22.5	26.3	24.9
GPT	(IU/l)	37	23～50	29.5	25.5	37.5	25.5	33	27.3	32.8	38.8
ALP	(IU/l)	72	40～103	145.5	107.2	157.1	172.1	65.5	81	64.3	51.2
γ-GTP	(IU/l)	41.0	17.7～64.2	25.87	17.76	27.78	32.07	37.57	39.61	38.68	34.38
Choline esterase	(IU/l)	187	102～272	190.5	201	169.4	201	163.5	149.7	176.6	164.2
Creatine kinase	(IU/l)	491	177～805	490.4	370.3	410.7	690.2	712.7	601.7	826.7	709.8
Total protein	(g/dl)	7.77	6.34～9.20	7.49	7.27	7.31	7.89	7.36	7.28	7.4	7.41
Albumin	(g/dl)	4.43	3.95～4.91	3.45	3.51	3.52	3.31	3.85	3.82	3.66	4.06
Globulin	(g/dl)	3.35	2.18～4.52	4.04	3.76	3.79	4.58	3.52	3.46	3.75	3.34
A/G		1.36	0.91～1.81	0.86	0.93	0.93	0.72	1.1	1.11	0.98	1.21
Total cholesterol	(mg/dl)	76	59～94	77.8	90.7	66.3	76.5	62	61.2	66.4	58.5
Triglyceride	(mg/dl)	34	16～64	40.5	34.4	54.7	32.4	22.6	18.1	21.7	28
Phospholipid	(mg/dl)	88	54～122	60.2	68.2	58.7	53.6	70.6	61.5	75.8	74.4
Glucose	(mg/dl)	77	66～88	97.5	89.6	117.2	85.6	106.9	127	103.7	89.9
Total bilirubin	(mg/dl)	0.24	0.17～0.31	0.22	0.25	0.2	0.2	0.22	0.22	0.21	0.24
BUN	(mg/dl)	10.3	5.8～14.8	11.77	11.09	13.01	11.21	11.81	10.75	12.44	12.23
Uric acid	(mg/dl)	0.01	0.00～0.03	0.023	0.02	0.03	0.02	0.023	0.02	0.02	0.03
Creatinine	(mg/dl)	1.98	1.51～2.45	2.07	1.93	1.96	2.32	1.86	1.98	1.78	1.82
Calcium	(mg/dl)	10.1	9.3～10.9	10.38	10.66	10.5	9.98	10.85	10.75	10.73	11.08
Inorganic phospharus	(mg/dl)	6.2	4.7～7.7	5.91	5.9	6.11	5.71	6.43	6.87	6.7	5.73
Na	(mEq/l)	147	141～152	141.4	142.3	141.6	140.2	142.9	142.3	144.3	142.2
K	(mEq/l)	4.73	3.81～5-65	4.08	4.02	4.11	4.12	3.52	3.27	3.41	3.88
Cl	(mEq/l)	103	96～111	98.7	98.6	97.8	99.6	94.9	94.1	95.5	95

Note) These data were obtained from sows around 18 months in age.

(LWD pigs, cited from reference #1 with permission)

progeny and conventionally bred pigs.

In three somatic cell cloned sows, no biological differences in reproductive performances including litter size, viability index and lactation index were found when these were compared those in conventionally bred sows [4] (Table C); however, body weights of these piglets were lighter than those derived from conventionally bred sows. When somatic cell cloned LWD sows were mated with conventionally bred Duroc boar, no biological differences in fetal growth were found when these were compared those derived from conventionally bred pigs [1] (Fig. G). Growth performances in progeny of cloned pigs seem to be not investigated.

Investigations for meat productive performances including body weight gain (Table D), carcass traits[5] (Tables E, F) were investigated with 44 progeny of somatic cell cloned pigs. More detailed analyses with Longissimus thoracis such as physicochemical properties [6] (Table G) and fatty acid composition[3] (Table H) were also investigated in 27 and 11 progeny of clones, respectively. These findings showed that the meat productive performances and meat quality in progeny of cloned pigs were practically equivalent to those derived from conventionally bred pigs. Meat productive performances in somatic cell cloned pig seem to be not reported.

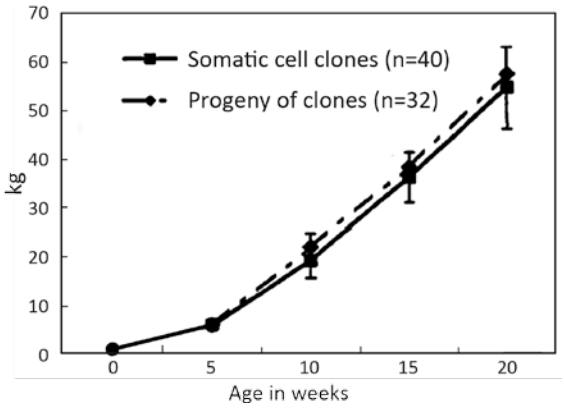

Fig. F. Growth curves observed in a somatic cell cloned pigs and their progeny
(Both sexes, Jin-Hua Pigs, cited from reference #3 with permission)

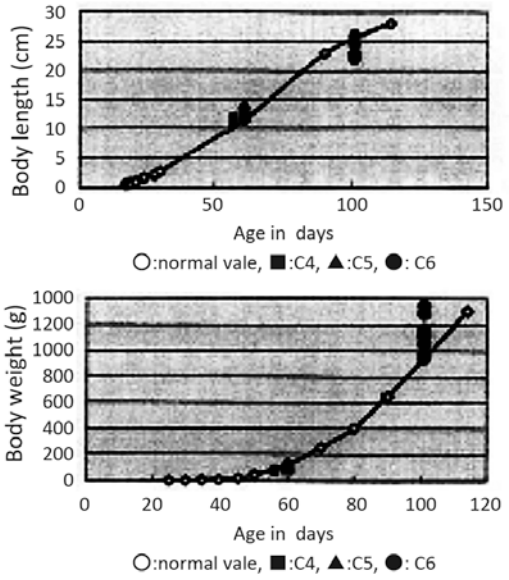

Fig. G. Growth of fetuses carried by progeny of clone (LWD) mated with conventionally bred boar (Duroc)
(Cross breed pigs, cited from reference #1 with permission)

Table C. Production of piglets from somatic cell cloned sows and conventionally bred sows

	Gestation period (days)	Litter size	Alive piglets at birth	Wearers	Survival rate at birth (%)	Average body weight of piglets (kg)	
						At birth	Three weeks after birth
S-1[a]	112	13	11	(10)	(90.9)	0.6	(2.53)
S-2	113	9	9	8	88.9	0.76	3.64
S-3	114	13	11	10	90.9	0.69	2.93
Average of cloned sows[b]	113	11.7	10.3	9	90	0.68*	3.25
Average of conventionally bred sow	113.8	11	10	9.5	95	0.88	4.15

a) Piglets were artificially suckled due to milking trouble of the sow.
b) A result excluding values shown in brackets.
* $P<0.05$.

(Jin-Hua pigs, cited from reference #4 with permission)

Table D. Birth weights and body weight gain in progeny of somatic cell cloned sows and conventionally bred sows in Jin-Hua pigs

Items	Number of piglets	Birth weight (kg)	Dairy gain (g)	
			Birth～30 kg	30～70 kg
Progeny of cloned sows	44	0.71±0.01^{A}	308.6±29.6^{A}	549±61.9^{A}
Conventionally bred piglets	21	0.91±0.06^{B}	314.6±18.4^{A}	444.0±91.1^{B}
Conventionally bred piglets of Duroc	27	1.60±0.07^{C}	403.8±42.8^{B}	867.7±150.6^{C}

^{A,B,C} Within each groups, rows with different superscripts differ. $P<0.001$.

(Jin-Hua pigs, cited from reference #5 with permission)

Table E. Carcass traits of progeny derived from somatic cell cloned pigs and conventionally bred pigs (1)

Items	Number of pigs	Carcass weight (kg)	Loin length II (cm)	Thickness of back fat				number of vertebrae
				Sholder	Back	Hip	Average	
Progeny of cloned sows	30	45.2±2.4^{a}	54.1±1.7^{A}	5.3±0.6^{A}	2.9±0.4^{A}	3.6±0.5^{A}	3.9±0.5^{A}	19.4±0.5^{Aa}
Conventionally bred pigs	19	45.7±6.3	56.0±1.1^{B}	5.2±0.6^{A}	2.8±0.4^{A}	3.3±0.5^{A}	3.8±0.5^{A}	19.7±0.5^{Bb}
Conventionally bred pigs of Duroc	27	46.7±3.1^{b}	57.6±1.7^{C}	3.2±0.5^{B}	1.8±0.4^{B}	2.8±0.4^{B}	2.6±0.4^{B}	21.1±0.4^{C}

^{A,B,C} Within each groups, rows with different superscripts differ. $P<0.001$.

^{a,b,c} Within each groups, rows with different superscripts differ. $P<0.05$.

(Jin-Hua pigs, cited from reference #5 with permission)

Table F. Carcass traits of progeny derived from somatic cell cloned pigs and conventionally bred pigs (2)

Items	Number of pigs	Rib eye area (cm^2)	Ratio of each retail cut among shoulder, loin/belly and ham (%)		
			Shoulder	Loin/belly	Ham
Progeny of cloned sows	30	9.8±1.4^{A}	31.4±0.9	40.6±1.3^{Aa}	27.9±1.6^{Aa}
Conventionally bred pigs	19	9.7±1.0^{A}	31.6±1.2	41.9±1.5^{Bb}	26.5±1.0^{Bb}
Conventionally bred pigs of Duroc	27	14.8±1.5^{B}	31.3±1.0	36.3±1.9^{C}	32.4±1.5^{C}

^{A,B,C} Within each groups, rows with different superscripts differ. $P<0.001$.

^{a,b,c} Within each groups, rows with different superscripts differ. $P<0.05$.

(Jin-Hua pigs, cited from reference #5 with permission)

Table G. Physiological properties of M. longissimus thoracis derived from somatic cell cloned pigs and conventionally bred pigs in Jin-Hua pigs

Items	Number of pigs	Drip loss (%)	Cooking loss (%)	Shear force value (lb/cm^2)	pH
Progeny of cloned sows	30	6.69	26.80^{A}	5.65^{a}	5.47^{A}
Conventionally bred pigs	19	7.56	24.89^{B}	5.44^{a}	5.56^{Bb}
Conventionally bred pigs of Duroc	27	7.41	28.99^{C}	7.38^{b}	5.67^{Cc}

^{A,B,C} Within each groups, rows with different superscripts differ. $P<0.001$.

^{a,b,c} Within each groups, rows with different superscripts differ. $P<0.05$.

(Jin-Hua pigs, cited from reference #6 with permission)

Table H. Fatty acid composition of fat in different fat tissues derived from progeny of somatic cell cloned pigs and conventionally bred pigs in Jin-Hua pig

Fat tissues	Pigs	Composition (%)					
		Myristic acid	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid
Outer layer of back fat	Progeny of clones	1.7±0.1	27.3±0.5	3.4±0.4	12.0±0.5	43.6±0.9	12.1±0.6
	(Range)	(1.6～1.9)	(26.3～28.1)	(3.0～4.1)	(11.2～12.7)	(42.0～45.0)	(11.2～13.2)
	Conventionally bred pigs	1.7±0.2	27.3±0.9	3.6±0.4	11.8±0.5	43.5±0.6	12.1±1.1
	(Range)	(1.3～2.0)	(25.6～28.8)	(2.9～4.2)	(11.1～12.8)	(42.3～44.4)	(10.4～13.9)
Inner layer of back fat	Progeny of clones	1.5±0.1	27.4±0.6	2.4±0.2[a]	15.5±0.5[A]	43.6±0.9	9.6±0.5
	(Range)	(1.3～1.7)	(26.3～28.5)	(2.0～2.7)	(14.6～16.4)	(42.1～44.8)	(8.9～10.7)
	Conventionally bred pigs	1.5±0.2	27.5±0.6	2.7±0.3[b]	14.6±0.7[B]	44.1±0.7	9.6±0.5
	(Range)	(1.3～1.8)	(26.5～28.9)	(2.3～3.2)	(13.4～16.0)	(43.0～45.5)	(8.8～10.7)
Perinephric fat	Progeny of clones	1.4±0.2	27.8±1.6	2.2±0.4	17.7±1.5	42.3±2.1	8.6±0.6
	(Range)	(1.1～1.7)	(24.2～29.3)	(1.4～2.7)	(15.6～19.9)	(38.6～44.9)	(8.0～9.9)
	Conventionally bred pigs	1.4±0.2	27.7±1.3	2.2±0.3	17.2±1.1	42.8±1.8	8.7±0.8
	(Range)	(1.0～1.7)	(24.8～29.3)	(1.4～2.6)	(15.9～19.0)	(39.7～46.2)	(7.6～10.1)
Loin fat	Progeny of clones	1.5±0.2	27.7±1.8	2.3±0.4	17.0±1.2	42.9±1.2	8.6±0.4
	(Range)	(1.2～1.8)	(24.5～30.0)	(1.6～3.0)	(15.5～19.0)	(40.8～44.4)	(7.9～9.1)
	Conventionally bred pigs	1.5±0.2	28.5±0.9	2.5±0.5	16.4±1.7	42.8±1.4	8.2±0.5
	(Range)	(1.2～1.8)	(26.6～29.7)	(1.8～3.2)	(14.1～19.5)	(39.2～44.7)	(7.7～9.3)

[A,B,C] Within each groups, rows with different superscripts differ. $P<0.01$.

[a,b,c] Within each groups, rows with different superscripts differ. $P<0.05$.

(Jin-Hua pigs, cited from reference #3 with permission)

4. Investigations concerning characteristics of animal products

Characteristics of animal products derived from progeny of somatic cell cloned pigs were investigated with 40 pigs [3]. In this investigation, detailed data including growth performance, blood investigation, macronutrients (water content, protein, lipid and ash content) of internal organs (liver, heart) and muscles (*longissmus thoracis* and *biceps femoris*), nucleic acid related materials (six materials including ATP) and fatty acid (six fatty acids including Myristic acid) of muscles (*longissmus thoracis* and *biceps femoris*) were obtained. Moreover, 28-day feeding study with mice (Fig. H), mouse abdominal wall method and mouse micronucleus test were carried out with freeze-dried meat powder derived from progeny of somatic cell cloned pigs. No biological differences in these findings were found when these were compared those obtained from conventionally bred pigs. Characteristics of animal products derived from somatic cell cloned pig seen to be not investigated.

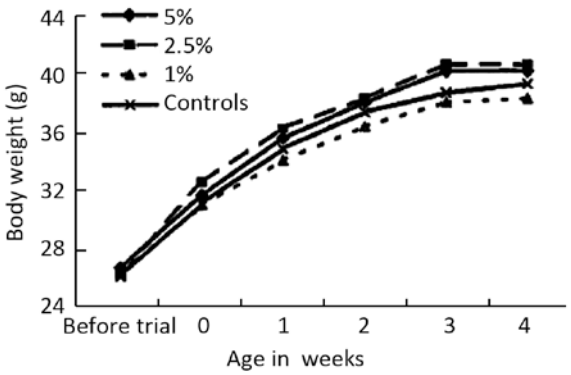

Fig. H. Body weight curves of male mice (ddY) fed diet supplemented with meat powder derived from progeny of somatic cell cloned pigs for 28 days
(Jin-Hua Pigs, cited from reference #3 with permission)

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体細胞クローン牛・後代牛の健全性ならびに生産物性状に関する国内調査報告

渡辺伸也

農研機構畜産草地研究所，つくば市，305-0901

摘　要

わが国において生産された体細胞クローン牛及びこれら由来の後代牛を中心に，個体としての健全性と生産物性状を調査した。特に，後代牛については，人工授精などによる生産効率が良好なため，産業利用上の実用性が高いと考えられることから，調査の早期実施が焦眉の問題であった。調査にあたっては，「先端技術を活用した農林水産研究高度化事業（農林水産省農林水産技術会議事務局）」の採択課題「産業利用に向けた体細胞クローン牛に関する技術開発と調査（課題番号：1602）」の後代牛を対象とした試験に加え，外部有識者の助言に基づく全国調査やこれまでに公表されている国内の成果資料の収集も実施した。これらの取組によって，諸外国の研究者や関係機関の要望が強いにもかかわらず，費用がかかるなどの理由から，実施例がない体細胞クローン後代牛の生産物性状調査をはじめ，国内関係機関の協力による体細胞クローン牛・後代牛の一般臨床検査と血液検査の全国調査（2005（平成 17）年 4 月に実施）による 63 頭の体細胞クローン牛（その当時，全国で繋養されていた牛の約 60%）と 25 頭の後代牛のデータ，転帰の全国調査（2006（平成 18）年 7 月実施）による 482 頭の体細胞クローン牛（その時点までに全国で生産された牛の 97.5%）と 202 頭の後代牛のデータを得た。また，収集した体細胞クローン牛・後代牛の健全性に関する成果資料を分析することで，出生後，24 時間以上生存した体細胞クローン牛の 51.6% に相当する 173 頭のクローン牛と 31 頭の後代牛の調査データを整理・分類できた。これらの臨床・病理（個体識別，血液性状，病理），成長・発育，繁殖性及び乳肉生産（搾乳，肥育）の広範な調査分野にわたるデータを分析した結果，生後 200 日以上，生存した体細胞クローン牛は，一般牛と同程度に生育し，一般牛と差異のない生理機能を有することが判明した。また，体細胞クローン後代牛についても，高度化事業（1602）で調査した 16 頭のデータを加え，体細胞クローン牛の場合と同様に検討した結果，データが存在するいずれの調査分野においても一般牛との差異は認められなかった。さらに，体細胞クローン牛及びその後代牛が生産した乳肉の生産性状調査において，栄養成分分析，アレルギー誘発試験（マウス腹壁法試験），消化試験（ラット），小核試験（マウス），飼養試験（ラット）の各検査で得られたデータを一般牛が生産した乳肉で得られたものと比較した結果，生物学的な差異は認められなかった。

キーワード：牛，体細胞クローン，後代，健全性，生産物性状

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編 集 委 員 会 事 務 局
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菊 池 幸 夫

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独立行政法人　農業・食品産業技術総合研究機構
畜産草地研究所

〒305-0901　茨城県つくば市池の台 2
TEL　029-838-8600(代)
FAX　029-838-8606

印刷所　　松枝印刷株式会社

畜産草地研究所研究報告及び畜産草地研究所研究資料投稿要領

13畜草B第43号
平成13年4月1日

（目的）
第１条　畜産草地研究所研究報告及び畜産草地研究所研究資料への投稿については，独立行政法人農業･食品産業技術総合研究機構刊行物著作権取扱規程（14規程56号）に定めるもののほかこの要領の定めるところによる。

（投稿者の資格）
第２条　投稿者は原則として，畜産草地研究所職員（以下「職員」という。）及び流動研究員，依頼研究員，日本学術振興会特別研究員，日本学術振興会外国人特別研究員等（以下「他の職員」という。）とする。
一　職員が投稿する内容は，主として畜産草地研究所（以下「研究所」という。）で行った研究とする。
二　他の職員が投稿する内容は，研究所で行った研究とする。

（投稿原稿の内容）
第３条　投稿原稿の内容は次のとおりとする。
1　畜産草地研究所研究報告（Bulletin of National Institute of Livestock and Grassland Science / 略誌名：Bull. Natl. Inst. Livest. Grassl. Sci.）
一　原著論文：研究所において行った試験研究及び研究所以外の者に委託して行った試験研究の成果に関わる論文とする。
二　短　　報：一以外の研究の予報，速報などの短報とする。
三　技術論文：新しい技術や技術の組立，実証などを主体とする報告。
四　総　　説：畜産草地研究に関わるものとする。総説は投稿のほか，編集委員会が依頼したものを含む。
五　学位取得論文：研究所において主として行った試験研究による学位取得論文とする。
2　畜産草地研究所研究資料（Memoirs of National Institute of Livestock and Grassland Science / 略誌名：Mem. Natl. Inst. Livest. Grassl. Sci.）
調査資料･技術資料･研究資料：研究所において行った試験研究及び研究所が研究所以外のものに委託して行った試験研究のうち，学術的･産業的に有用な未発表の資料とする。

（原稿の執筆）
第４条　原稿の執筆にあたっては，別に定める畜産草地研究所研究報告及び畜産草地研究所研究資料執筆要領（13畜草Ｂ第44号）に基づくものとする。使用する言語は日本語又は英語とする。

（原稿の提出）
第５条　次の手続きにより原稿及び原稿提出票を事務局に提出する。
一　職員は原稿提出票に必要事項を記載し，所属研究チーム長及び担当する研究管理監等の校閲を受ける。
二　他の職員は原稿提出票に必要事項を記載し，所属研究チーム長及び研究チームを担当する研究管理監等の校閲を受ける。

（受付）
第６条　原稿及び原稿提出票を事務局が受け取った日を受付日とする。受理日は編集委員会の審査の結果，掲載が妥当と認められた日とする。

（審査）
第７条　編集委員会は次の手続きにより論文を審査する。ただし，学位取得論文については審査を省略することができる。
一　編集委員会は論文の内容により審査員正副をそれぞれ１名決定し，論文審査を依頼する。審査員は研究所内及び研究所外の研究者等とし，その氏名は公表しない。
二　審査員は論文審査票により審査を行う。また必要に応じて指摘事項を書き出し提出する。
三　事務局は審査員と著者の間のやり取りの対応にあたる。
四　編集委員会は審査員の審査結果を参考にして掲載の可否を判断する。
審査の内容によっては著者に原稿の訂正を求めることができる。
五　著者は審査結果を受領後，編集委員会が指定する期日までに修正原稿を事務局に提出する。

（校正）
第８条　著者による校正は原則として初校のみとする。校正は誤植の訂正程度にとどめる。やむを得ず大きな変更等を行う場合には編集委員会の承認を得なければならない。

（別刷り）
第９条　別刷りは次のとおりとする。
一　100部とし，筆頭著者が代表で受け取る。
二　別刷りの追加を希望する場合は研究チーム負担で印刷する。

附　　則
この規定は，平成14年４月１日から施行する。

附　　則
この規定は，平成15年10月１日から施行する。

附　　則
この規定は，平成18年４月１日から施行する。

附　　則
この要領は，平成20年４月１日から施行する。