production is approximately 30 percent and, considering the impact of environmental conditions on milk production, suggest that standardizing individual feeding conditions may be helpful for future comparisons.

Table VI-24: Milk Composition (mean ± standard deviation) in First and Second Lactations
(adapted from Yonai et al. 2005)

	Milk Yield	Fat (%)	Fat (kg)	Protein (%)	Protein (kg)	SNF* (%)	SNF (kg)
Jersey (n=4)							
First lactation	5,896.4 ± 332.0	5.0 ± 0.2	300.3 ± 23.9	3.8 ± 0.2	225.5 ± 20.4	9.4 ± 0.2	560.0 ± 47.8
Donor animal	5,064.0	4.9	242.3	4.0	197.1	9.6	477.2
Second lactation	7,262.8 ± 222.6	5.13 ± 0.13	375.3 ± 26.2	3.78 ± 0.10	274.8 ± 13.4	9.35 ± 0.10	681.3
Donor animal	6,087.0	4.6	280	3.67	224	9.30	566
Holstein (n=6)							
First lactation	9,333.0 ± 476.4	4.7 ± 0.1	440.3 ± 36.7	3.3 ± 0.1	304.2 ± 27.6	9.0 ± 0.1	835.5
Donor animal	10,968.0	4.1	452.0	3.3	359.0	—	—
Second lactation	11,271.4 ± 1084.7	4.5 ± 0.2	510.5 ± 53.4	3.1 ± 0.1	353.5 ± 31.4	8.7 ±0.1	978.7
Donor animal	11,442.0	3.9	446.2	2.8	320.4	—	—

* SNF is milk solids not fat

Heyman et al. (2007) studied milk fat composition in five cattle clones and five of their comparators. Milk samples were collected at days 80 and 180 during the first lactation. Comparisons were limited in this study to milk from comparators vs. milk from clones; no comparisons to historical reference values were reported. At both time points, the proportion of stearic acid was lower in the milk from the clones. In addition, two indices of delta-9 desaturase activity, C18:0 and C18:2-*c*9-*t*11, were higher in the milk from the clones compared to milk from control cows. The proportion of C18:*t*11 was also lower in clones, but only at the 180 day time point. As all five clones in this study were of the same genotype, the authors speculate that differences in delta 9 desaturase activity may reflect a within breed genotypic difference. Milk composition data from clones of more genotypes are needed to confirm these results.

In the most comprehensive analysis of milk composition from clone cows published to date, Laible et al. (2007) collected milk samples from nine clones (six Friesian and three Jersey) and five sexually-derived cows (3 Friesian and 2 Jersey) during early, mid, and late lactation. Because, in general, milk from Jerseys has higher concentrations of protein, casein and fat compared to milk from Holstein or Friesian cows, the authors compared results within each breed, rather than across all breeds. That is, for each constituent assayed, data were presented for Friesian controls (n=3), Jersey controls (n=2), two lines of Friesian clones (n=3 per line) and Jersey clones (n=2). In addition to comparing milk from clones and non-clones, the authors made comparisons with published reference ranges. The compositional analysis in this study included all of the proximates identified in Table VI-16 as useful for showing no material differences between milk from clone and comparator cows (vitamins, minerals, fatty acid profiles, amino acid profiles, and carbohydrate (lactose)).

Proximate analyses indicated that the ranges of concentrations of fat, protein, casein, lactose, and pH in milk from clones were all within normal ranges for milk from conventional, pasture-fed dairy cattle in New Zealand (Mackle et al. 1997; Mackle et al. 1999; Auldist et al. 2004). The authors state that there were no differences between milk from control and clones in the percentages of 24 fatty acids, or in the total percentages of saturated, monounsaturated or polyunsaturated fatty acids. For each group of clones, mean values for three to six individual fatty acids in milk were outside the respective reference ranges. These observations were not unique to the clones, as values for three individual fatty acids in controls were also outside the reference ranges. Concentrations of minerals (calcium, magnesium, phosphorus, potassium, selenium, sodium and zinc) in milk from clones cows were similar to concentrations in comparator samples and within reference values, with the exception of potassium in Jersey controls (116 ± 10 mg/100 g vs. reference range of 126-173 mg/100g) and selenium in one line of Friesian clones (0.4 ± 0.2 mg/100 g vs. reference range of 0.6-2.0 mg/100 g). For 18 amino acids and vitamins (A, B_2, B_{12}), most of the values (57/90 and 13/15, respectively) were outside the reference ranges, but there were no dramatic differences between values for clones and their breed-matched controls.

In their Discussion, the authors point out that the discrepancies between the observed values and reference ranges may be explained by the small numbers of animals in their study, and that comparison of milk from clones to milk from a wider selection of control animals that represent more genotypes, nutrition and farming systems would put the values from clones within reference ranges for normal bovine milk. Thus from this pilot study, the authors conclude that the composition of milk from the nine clones was "broadly similar" to milk from the five comparator cows.

ii. The Report of the Japanese Research Institute for Animal Science in Biochemistry and Toxicology

The Japanese Research Institute for Animal Science in Biochemistry and Toxicology provided a report entitled "*Investigation on the Attributes of Cloned Bovine Products*" published by the Japan Livestock Technology Association (Japan 2002: Appendix I). CVM was able to obtain a seven page English-language summary translation of the original 489 page Japanese report. Only the English-language summary is reviewed in this risk assessment.

The study investigated blood, milk, and meat constituents in blastomere nuclear transfer clones (BNT) and SCNT cattle clones. No information was provided on the production of the BNT or SCNT clone cattle in the English translation, and the comparator group was identified as "ordinary cattle." The results for milk composition are discussed in this section; results for meat composition are discussed in the section entitled Compositional Data on Meat from Clones. In addition, the Japanese report includes results of rodent feeding studies conducted with edible products derived from the cattle clones. These and other studies are discussed below in the section entitled Allergenicity and Feeding Studies in Rodents.

Milk constituents were compared between ordinary cattle, BNT clones, and SCNT clones. The results are reported as the mean of samples obtained three and six weeks after parturition and provided in Table VI-25. No biologically significant differences were observed between any of the groups for the parameters tested.

Table VI-25: Milk Constituents in BNT and SCNT Clones and Ordinary Cattle
(from Japan 2002)

Classification	Cattle No.	Protein	Fats	Sugars	Ash content	Water content	Calcium	Cholesterol
	g/100 g						mg/100 g	
Ordinary cattle	Min. value	3.0	2.2	4.6	0.7	88.1	100	8
	Max value	3.4	3.3	4.6	0.7	89.7	110	10
	Mean value	3.3	2.7	4.6	0.7	88.9	105	9
BNT clones	No.1	2.9	2.3	3.0	0.8	91.1	95	9
	No.2	2.9	3.6	3.5	0.7	89.3	105	9
SCNT clones	No 1	3.1	4.3	4.6	0.7	87.4	120	9
	No.2	3.3	2.6	4.4	0.7	89.1	115	11
	No.3	3.3	3.1	4.5	0.7	88.5	115	10

iii. Summary Statement on Composition of Milk from Clones

Several peer-reviewed studies describe the composition of milk from bovine clones. In addition to gross composition (percent solids, fat, protein, and lactose), some reports include a detailed analysis of fatty acids, vitamins, minerals, and amino acids, and in some cases, comparisons are made with previously published reference values for milk composition. These studies indicate that milk from cow clones is not significantly different in composition from milk from non-clones. Some minor differences have been identified in the composition of milk from clones compared to non-clones or reference values, but in each of these reports, the authors attribute the minor differences to diet, environmental conditions, small numbers of animals, and limited numbers of genotypes, rather than to cloning *per se*. None of these differences, however, indicate the presence of hazards that could pose food consumption risks, as they all fall within published historical values for milk. We therefore that milk derived from bovine clones does is not materially different from milk from milk from conventionally bred cattle.

d. Characterization of Meat from Clones and Their Progeny

i. Cattle

Peer-reviewed Reports

Two early linked reports on carcass merit[106] (*e.g.,* dressing percentage, fat depth, rib-eye area, yield and quality grade) of cattle produced via BNT have been published (Diles et al. 1996a,b). Neither paper addresses food safety issues. Both papers evaluate the degree to which body measurements are heritable (Diles et al. 1996a), and the degree to which there is phenotypic variability among clones and closely related siblings. The studies conclude that animals derived from BNT provide good models for determining which traits have strong genetic correlations.

The study by Tian and her colleagues at the University of Connecticut discussed previously for milk composition also reports the results of studies on the composition of meat from bovine SCNT clones (Tian et al. 2005). Cultured skin fibroblasts or cumulus cells were used to clone an adult Japanese Black beef bull, selected as a superior breeding stud with superior marbling traits at 17 years of age. Six bull clones were produced; four survived and were apparently normal. The clone bulls were maintained on the same diet and raised in the same facility with eight

[106] Carcass merit programs have been initiated by a group of academics and beef producers to correlate bovine genetics and phenotypic markers for consumer-desired traits such as marbling, tenderness, and composition. Because consumers desire consistency in meat products, producers demonstrating that their herds have good performance and carcass data can leverage higher market prices for their beef. Identification of genetically determined traits can also lead to selective breeding programs that improve herd meat quality in a directed manner.

comparator non-clone animals which were produced by artificial insemination using semen from the son of the original donor bull. The "genetically" matched comparators therefore shared 25 percent of their genetic makeup with the clones. In addition, 20 age-matched sexually reproduced Japanese Black beef cattle were used as breed comparators to establish the normal range for each measured parameter. All bulls were castrated at 3 months of age and raised on standard growing ration from 8 to 26 months of age. The comparators and two of the clones were slaughtered and subjected to standard meat analyses. Variables measured included the following:

- Organ or body part weights
- Total proportion of meat and fat in the dressed carcass
- Cross section of the left dressed carcass between the 6^{th} and 7^{th} rib
- Moisture in six muscles (infraspinatus, longissimus thoracis, latissimus dorsi, adductor, biceps femoris, and semitendinosus)
- Crude protein in six muscles (infraspinatus, longissimus thoracis, latissimus dorsi, adductor, biceps femoris, and semitendinosus)
- Crude fat content in six muscles (infraspinatus, longissimus thoracis, latissimus dorsi, adductor, biceps femoris, and semitendinosus)
- Fatty acid composition (lauric acid, myristic acid palmitic acid palmitoleic acid, stearic acid, oleic acid, linoleic acid and linolenic acid) of five major fat tissues (subcutaneous fat, inter-muscular fats, celom fat, and kidney leaf fat)
- Amino acid composition of the longissimus thoracic muscle
- Histopathology of all organs

The 90 percent confidence intervals for each parameter were compared in a paired analysis between the clone and non-clone genetic comparators. There were 12 instances where the clones and genetic comparators showed statistical differences:

- Amount of mesentery fat
- Proportion of longissimus thoracic muscle over body weight
- Muscle moisture
- Amount of crude protein in the semitendinosus muscle
- Amount of linolenic acid in the kidney leaf fat
- Amount of linolenic acid in the longissimus thoracis
- Amount of linolenic acid in the semitendinosus muscles
- Amount of oleic acid in the semitendinosus muscle
- Amount of palmitic acid in the semitendinosus muscle
- Amount of linoleic acid in the semitendinosus muscle

All of the measurements were higher in the clones than in the genetic or breed comparators, except for crude protein or muscle moisture in semitendinosus muscle. The differences in mesentery fat and fatty acid content were attributed to the characteristics of the donor bull (superior marbling). It is noted that the clones had a marbling score of 8 out of 12, compared to an industry standard of 5.2, and genetic comparator score of 6.5. All of the other variables fell within normal industry standards. The researchers conclude that the meat from somatic animal clones falls within normal industry standards and does not significantly differ from those of the genetic or breed comparators. The differences observed were considered due to the superior genetics of the donor bull from which the line of clones was derived. No abnormalities were reported in the pathology or histopathology for tissues collected from the clones.

This group has also reported meat composition in 11 cattle clones and 11 sexually-derived comparators (Yang X et al. 2007b). (Blood chemistry and hematological parameters in these animals have been discussed previously in the Post-pubertal Maturation Developmental Node.) Ages ranged from ">12 months" to 43 months. There were six females and five males in each group representing several breeds (Angus, Brangus, Holstein, Red and White Holstein, and cross breeds). No anatomical or other obvious defects were observed in carcasses after slaughter. Mean values for meat composition, amino acids, fatty acids, vitamins, minerals, cholesterol, and proximate analyses of water, fat, protein and carbohydrate are presented. No differences were detected between clones and comparators for any of the constituents analyzed. In addition to differences between mean values, the authors also analyzed the standard deviations of the means and found that for essentially all traits examined, standard deviations were similar between clones and non-clones. Moreover, statistical outliers were no more likely to occur in the clone group. This finding indicates that variability in meat composition in beef cattle clones is not different from that in sexually–derived comparators.

Citing the fact that meat quality is influenced by both contractile and metabolic characteristics of muscle, Heyman et al. (2007) evaluated muscle characteristics in nine heifer clones and eight comparator heifers. Samples of semitendinosus muscle were obtained by biopsy at 8, 12, 18 and 24 months of age[107].The findings of this study are summarized textually; no actual data are provided. At eight and 12 months, the proportions of myosin heavy chain (MyHC) I (a slow oxidative isoform of a key muscle protein), and MyHC IIa (a fast oxido-glycolytic isoform of the same key muscle protein) were significantly higher in clones compared to comparators, while the proportion of MyHC IIb (another fast glycolytic isoform) was lower in clones. At the same time points, oxidative metabolism, measured by isocitrate dehydrogenase activity (ICDH), was significantly higher in clones compared to comparators. Glycolytic metabolism (the mechanism

[107] Only a summary of results is presented in this report; no actual data are shown. Many of these results were published in INRA reports in 2004 and 2006. Because these reports are not readily available via PubMed, the results are also discussed here.

by which muscles break down stored carbohydrates), measured by lactate dehydrogenase (LDH) activity, was similar between clones and controls. No differences in the proportions of myosin heavy chains, ICDH, or LDH were found between clones and controls at 18 or 24 months. The authors interpreted these findings as evidence that clones had slower muscles associated with more oxidative metabolism compared to the comparators, and that the contractile and metabolic profile of muscle from clones corresponded to a delay in muscle differentiation at 8 and 12 months of age. This conclusion is based solely on biochemical data; no corresponding histological or physiological studies are reported.

In the same muscle samples used to evaluate contractile and metabolic parameters, Heyman et al. (2007) also determined the fatty acid composition. At eight months of age, clones had a higher proportion of polyunsaturated fatty acid (37.2 percent vs. 28.4 percent in controls) and a lower proportion of monounsaturated fatty acid (18.7 percent vs. 24.7 percent in controls). These differences may be related to the lower marbling in meat from clones compared to controls. At 24 months of age, clones had lower proportions of stearic acid, C18:1-*t*11 and C18:1-*c*11 than controls. At 18 and 24 months of age, clones had higher indices of delta-9 desaturase activity, but only for the C:16 index.

It is interesting that the alterations in fatty acid composition and delta-9 desaturase activity in meat from clones were also observed in milk from clones (Heyman et al., 2007; see section on Characterization of Milk from Cow Clones). Taken together, these results support the authors' conclusion that lipid metabolism may be altered in their population of cloned cattle. It is important to note, however, that comparisons in these studies were limited to clones vs. comparators (n = 5 to 9 per group), and no comparisons were made with historical reference values for either milk or meat. Clearly, similar studies using larger numbers of animals representing multiple genotypes are needed to verify these results.

The Report of the Japanese Research Institute for Animal Science in Biochemistry and Toxicology

Table VI-26: Meat Constituents in BNT and SCNT Clones and Ordinary Cattle
(from Japan 2002)

Classification	Cattle No.	Protein (g/100 g)	Fats (g/100 g)	Sugars (g/100 g)	Ash content (g/100 g)	Water content (g/100 g)	Cholesterol (mg/100 g)
Ordinary cattle	Min. value Max value	17.8 19.6	13.8 22.9	0.4 0.8	0.9 1.0	58.0 64.8	50 68
	Mean value	18.4	19.3	0.6	0.9	60.8	59
BNT clones		17.4	21.2	0.4	0.9	60.2	56
SCNT clones		16.8	23.8	0.5	0.9	57.9	68

As discussed in the section on milk composition, the Japanese Research Institute for Animal Science in Biochemistry and Toxicology provided an unpublished bound report "Investigation on the Attributes of Cloned Bovine Products" by the Japan Livestock Technology Association (Japan 2002).[108] The results for meat are discussed in this section. Takahashi and Ito (2004) have published a summary of these data, including some information characterizing the clones and their comparators. SCNT and BNT clones were derived from Japanese Black cattle at the Nara Prefectural Animal Research Center. Comparator animals were selected as conventionally bred Japanese Black cattle. All animals used for compositional analysis were sacrificed between 27 and 28 months of age, after fattening.

Meat constituents were compared between ordinary cattle, BNT clone cattle, and SCNT clone cattle. The results are reported as the mean of analytical samples obtained from 9 sites; shoulder, chuck loin, rib loin, loin end, brisket, round, silver side, rump, and tender loin, and are provided in Table VI-26.

No biologically significant differences were observed between any of the groups of cattle (ordinary cattle, BNT clone cattle, and SCNT clone cattle) for the parameters tested.

Cyagra dataset

Cyagra, the cloning company that provided the extensive physiological data discussed earlier in the risk assessment, also provided meat composition data. Eleven clones (6 female, 15 to 43 months; 5 male, 12 to 17 months) and an equal number of comparator cattle (over 12 months) were selected for the study. All animals were fed a standard ration for 30 days prior to slaughter. Samples (500 g each) were obtained of chuck arm roast, bottom sirloin tip roast, and short loin for analysis by an independent laboratory.

No biologically significant differences are observed in the composition of meat from clones and comparators. The results of the compositional analysis summarized across gender and cuts of meat are summarized in Table VI-27. A detailed presentation of the results is provided in Appendix E, the Cyagra Dataset.

[108] Some of these data are presented in Takahashi and Ito 2004; however, we have cited the original report as the data reporting is more complete.

Table VI-27: Meat Composition from Cyagra Clones and Comparators

Meat Analysis		**Overall Comparison**			
Sample Number					
Marked ID		**Clone**		**Comparator**	
Analyte	**Units**	**Mean**	**Std dev**	**Mean**	**Std dev**
Crude Fat	%	11.62	10.08	8.62	8.10
Moisture	%	66.18	7.68	68.57	5.51
Protein – Combustion	%	20.69	2.96	21.72	2.58
Protein – Kjeldahl	%	20.74	2.90	21.58	2.51
Ash	%	1.03	0.17	1.05	0.13
Balance (protein+moist+ash+fat)	%	99.56	1.72	99.82	0.89
Amino Acid Profile (results below)					
Tryptophan	%	0.25	0.03	0.26	0.03
Aspartic Acid	%	1.96	0.31	2.08	0.23
Threonine	%	0.93	0.15	1.01	0.12
Serine	%	0.79	0.14	0.86	0.12
Glutamic Acid	%	3.22	0.54	3.33	0.71
Proline	%	0.97	0.21	0.91	0.16
Glycine	%	1.08	0.27	1.08	0.21
Alanine	%	1.28	0.21	1.36	0.18
Cystine	%	0.23	0.04	0.24	0.04
Valine	%	0.89	0.21	1.07	0.14
Methionine	%	0.54	0.09	0.56	0.08
Isoleucine	%	0.81	0.20	0.98	0.12
Leucine	%	1.61	0.27	1.78	0.20
Tyrosine	%	0.69	0.11	0.74	0.08
Phenylalanine	%	0.84	0.14	0.91	0.10
Histidine	%	0.70	0.12	0.77	0.11
Lysine, Total	%	1.77	0.31	1.98	0.23
Arginine	%	1.33	0.23	1.41	0.17
Hydroxyproline	%	0.17	0.07	0.16	0.07
Fatty Acid (results below)					
C14:0 Tetradecanoic (Myristic)	%	0.28	0.24	0.23	0.24
C14:1 Tetradecenoic (Myristoleic)	%	0.15	0.17	0.09	0.10
C15:0 Pentadecanoic	%	0.04	0.04	0.04	0.04

Table VI-27: Meat Composition from Cyagra Clones and Comparators

C15:1 Pentadecenoic	%	0.00	0.00	0.00	0.00
C16:0 Hexadecanoic (Palmitic)	%	2.65	2.29	2.04	2.00
C16:1 Hexadecenoic (Palmitoleic)	%	0.69	0.68	0.45	0.44
C16:2 Hexadecadienoic	%	0.08	0.08	0.06	0.06
C17:0 Heptadecanoic (Margaric)	%	0.10	0.08	0.09	0.09
C17:1 Heptadecenoic Margaroleic	%	0.11	0.11	0.08	0.08
C18:0 Octadecanoic (Stearic)	%	1.18	0.93	1.05	1.10
C18:1 Octadecenoic (Oleic)	%	4.94	4.49	3.43	3.34
C18:2 Octadecadienoic (Linoleic)	%	0.31	0.24	0.22	0.18
C18:3 Octadecatrienoic (Linolenic)	%	0.05	0.05	0.05	0.05
C18:4 Octadecatetraenoic	%	0.07	0.08	0.04	0.04
C20:1 Eicosenoic (Gadoleic)	%	0.03	0.04	0.02	0.03
C20:4 Eicosatetraenoic (Arachidonic)	%	0.01	0.01	0.01	0.01
Total Monounsat. Fatty Acids Calc.	%	5.92	5.42	4.08	3.93
Total Polyunsat. Fatty Acids Calc.	%	0.54	0.44	0.40	0.33
Saturated Fatty Acids	%	4.25	3.51	3.47	3.42
Total Fat (as triglycerides)	%	11.24	9.76	8.34	7.99
Calcium	mg/100g	12.01	13.78	14.30	13.67
Iron	mg/100 g	2.29	0.74	2.32	0.71
Phosphorus	mg/100 g	179.09	27.31	191.21	28.12
Zinc	mg/100 g	3.86	0.67	4.14	0.64
Cholesterol	mg/100g	64.92	7.79	68.43	8.64
Niacin	mg/100 g	4.96	1.18	5.00	1.07
Vitamin B1 - Thiamine Hydrochloride	mg/100 g	0.10	0.04	0.09	0.02
Vitamin B2 – Riboflavin	mg/100 g	0.24	0.05	0.29	0.04
Vitamin B6	mg/100 g	0.33	0.08	0.37	0.11
Vitamin E	IU/100g	0.50	0.15	0.44	0.15
Hydroxyproline	%	0.17	0.07	0.16	0.07

ii. Swine

(a) Clones

ViaGen, Inc., worked in consultation with CVM to design two studies comparing the composition of meat from swine clones vs. age-matched, genetically related, AI-derived comparator animals. Experimental design, raw data, and CVM's analysis of the data are provided

in Appendix F, The ViaGen Dataset. Meat composition data were available for five clones (four Hamline and one Duroc) and 15 comparator animals (all Hamline). There were no differences between the Duroc and Hampshire clones, so data for clones were pooled.

Carcass characteristics were provided on four Hamline clones and 15 comparator barrows and are summarized in Table VI-28. The Duroc clone barrow carcass was condemned at slaughter due to a lung adhesion, and thus data relating to growth and carcass characteristics were not included for these parameters. In some cases of lung adhesions due to bacterial infection, animals fail to thrive, thereby affecting their growth rate and carcass characteristics – this was considered to be the case for the Duroc clone. Two other clones were approximately 100 pounds lighter than any of the other animals in the experiment at the time of slaughter, and for this reason were excluded from carcass evaluation. Hot carcass weights averaged 189.0 and 199.5 pounds for clone and comparator barrows, respectively. Carcass lengths were 82.4 and 84.5 cm for clones and comparators, respectively. Dressing percentages were 70.1 and 70.2 percent for clones and comparators, and were similar across groups. Backfat thickness over the first rib, tenth rib, last rib, and lumbar vertebra were slightly greater for comparator barrows than for clone barrows which may, in part, be due to the heavier body weight of comparator barrows at the time of slaughter.

Qualitative characteristics including USDA carcass muscle score, color, firmness, and marbling were similar across breeding regimens and are illustrated in Table VI-29. All animals received score 2 for carcass muscle. All of the clone and comparator barrows had marbling scores of either 1 or 2.

Table VI-28: Comparison of the Carcass Characteristics of Barrows Derived by Somatic Cell Nuclear Transfer (Clones) or Conventional Breeding (Mean ± standard deviation)
(from ViaGen, Inc.)

	Clones (n=4)	**Conventionally Bred (n=15)**
Hot Carcass Weight (lbs)	189.0 ± 13.8	199.5 ± 13.7
Carcass Length (cm)	82.4 ± 1.5	84.5 ± 2.7
Dressing Percentage (%)	70.1 ± 0.8	70.2 ± 1.4
Back fat Thickness (mm)		
First Rib	35.3 ± 2.1[b]	38.7 ± 3.1[a]
Tenth Rib	18.5 ± 3.1	22.2 ± 4.9
Last Rib	20.5 ± 4.7	23.3 ± 3.4
Last Lumbar	17.3 ± 3.2	21.0 ± 3.1
Loin Eye Area (cm^2)	44.0 ± 4.4	45.8 ± 4.0

Measurements of pH at 24 hours post-slaughter on the longissimus muscle were similar. Loin eye area for meat cuts for clone and comparator barrows were only slightly different at 45.8±4.0

and 44.0 ± 4.4 inches, respectively. The Hunter L*, a* and b* values were only slightly different between the groups of animals with the meat from clones being slightly darker and more red than meat from comparator barrows.

Table VI-29: Comparison of the Qualitative Carcass Characteristics of Barrows Derived by Somatic Cell Nuclear Transfer (Clones) or Conventional Breeding (Means ± standard deviation)
(from ViaGen, Inc.)

	Clones (n=4)	**Conventionally Bred (n=15)**
Longissimus pH at 24 hours	5.6 ± 0.1	5.7 ± 0.1
Carcass Muscle Score	2.0 ± 0.0	2.2 ± 0.40
NPPC Quality Scores		
Color	3.0 ± 0.0	2.7 ± 0.6
Marbling	1.5 ± 0.6	1.9 ± 0.5
Firmness	3.5 ± 0.6	2.9 ± 0.9
Hunter Color		
L*	52.2 ± 2.0	56.3 ± 4.4
a*	9.5 ± 1.4	7.6 ± 1.2
b*	17.6 ± 0.7	16.9 ± 1.2

Meat composition data were available for five clones (four Hamline and one Duroc) and 15 comparator animals (all Hamline). There were no differences between the Duroc and Hamline clones, so data for clones were pooled. Means ± standard deviations for fatty acids, amino acids, cholesterol, minerals and vitamins measured are presented in Table VI-30. Differences in individual analytes for clones and comparators were very small and not biologically relevant. Values for niacin and vitamin B_{12} in both clones and control swine were above USDA values for a similar type of swine muscle (shoulder blade and loin). Values for cholesterol and vitamin B_6 were similar to the USDA values.

Carcass qualitative characteristics were similar for clones and comparators. Differences in backfat thickness and marbling may be due to the lighter weight of clones at slaughter vs. comparators. Differences in meat nutrient composition were very small and likely not biologically relevant. No biologically relevant differences were observed in the food composition values between muscle of swine clones and comparators.

Table VI-30: Compositional Analysis of Meat from Swine Clones[1]
(from ViaGen, Inc.)

Component	Clones	Comparators
Amino acids (g)		
Alanine	1.26 ± 0.04	1.30 ± 0.04
Arginine	1.41 ± 0.03	1.47 ± 0.04
Aspartate	2.55 ± 0.28	2.43 ± 0.19
Cystine	0.25 ± 0.03	0.26 ± 0.02
Glutamate	3.41 ± 0.11	3.46 ± 0.09
Glycine	0.98 ± 0.04	1.02 ± 0.10
Histidine	0.99 ± 0.05	1.03 ± 0.05
Isoleucine	1.04 ± 0.05	1.05 ± 0.03
Leucine	1.74 ± 0.05	1.79 ± 0.04
Lysine	1.91 ± 0.06	1.96 ± 0.04
Methionine	0.54 ± 0.06	0.58 ± 0.03
Phenylalanine	0.86 ± 0.02	0.89 ± 0.02
Proline	0.85 ± 0.03	0.90 ± 0.06
Serine	0.90 ± 0.03	0.92 ± 0.02
Threonine	1.11 ± 0.04	1.14 ± 0.03
Tyrosine	0.77 ± 0.02	0.79 ± 0.02
Valine	1.10 ± 0.05	1.12 ± 0.04
Fatty Acids[2] (g)		
14:0	0.09 ± 0.06	0.05 ± 0.03
16:0	1.31 ± 0.82	0.95 ± 0.49
16:1	0.09 ± 0.04	0.14 ± 0.05
17:0	0.01 ± 0.01	0.00 ± 0.01
17:1	0.01 ± 0.01	0.00 ± 0.01
18:0	0.66 ± 0.41	0.55 ± 0.27
18:1	1.84 ± 0.84	1.49 ± 0.50
18:2	0.26 ± 0.08	0.19 ± 0.06
18:3	0.01 ± 0.01	0.00 ± 0.01
20:0	0.01 ± 0.01	0.00 ± 0.01
20:1	0.05 ± 0.03	0.04 ± 0.02
20:2	0.01 ± 0.01	0.01 ± 0.01
22:6	0.02 ± 0.03	0.01 ± 0.01
Cholesterol (mg)	55.5 ± 6.95	52.81 ± 2.69
Minerals (g)		
Calcium	0.004 ± 0.000	0.005 ± 0.003
Phosphorus	0.20 ± 0.01	0.21 ± 0.01
Iron	0.001 ± 0.000	0.001 ± 0.001
Zinc	0.002 ± 0.000	0.001 ± 0.000
Vitamins		
Niacin (mg)	10.90 ± 0.83	11.16 ± 1.58
B_6 (mg)	0.41 ± 0.09	0.48 ± 0.12
B_{12} (mcg)	0.21 ± 0.28	0.00 ± 0.00

[1]Data expressed as quantities per 100 g of homogenized meat.
[2] Data presented reflect those fatty acids with detectable levels in pork.

(b) Swine Clone Progeny

ViaGen also provided CVM with data from a study comparing the quality and composition of meat derived from the progeny of swine clones and sexually-derived swine. The data from this study, which was performed at USDA's Meat Animal Research Center, Clay Center, Nebraska, have subsequently published in the peer-reviewed literature (Walker et al. 2007). Sexually derived offspring of clones (n=242) were generated by artificially inseminating conventionally bred gilts with semen from four sires that were clones. Control offspring (n=162) were produced by artificially inseminating conventionally bred gilts with semen from three conventionally bred boars. Litters (n=61) of offspring of clones and control offspring were reared using the same management procedure. Pigs were slaughtered when their body weight reached approximately 123 kg. In addition to carcass characteristics, loin samples were collected and assayed for 58 different nutrients including numerous amino acids and fatty acids, vitamins (B_{12} and $B_{6,}$ niacin) and minerals (iron, phosphorus and zinc).

Carcass characteristics for the progeny of clones and their comparators are provided in Table VI-31, and discussed in more detail in Appendix F.

Table VI-31: Carcass Characteristics for Progeny Derived from Clone Boars or Conventionally Bred Boars (Means ± standard deviation) *(from ViaGen dataset, Appendix F)*

	Hampshire Comparator	**Hampshire Clone**	**Duroc Comparator**	**Duroc Clone**
Hot Carcass Weight (lbs)	176.2 ± 8.6	175.0 ± 8.7	173.9 ± 9.5	179.0 ± 9.1
Carcass Length (cm)	82.7 ± 2.2	81.6 ± 2.1	82.3 ± 2.2	81.5 ± 2.3
Loin Eye Area (cm^2)	6.7 ± 0.8	6.8 ± 0.8	6.6 ± 0.8	7.2 ± 0.9
Back fat Thickness (mm)				
First rib	22.2 ± 4.2	23.4 ± 4.4	23.8 ± 4.1	25.9 ± 4.2
Last rib	16.0 ± 2.9	16.9 ± 3.2	17.4 ± 2.4	19.0 ± 2.8
Last Lumbar	16.6 ± 3.4	17.0 ± 3.2	18.1 ± 2.6	19.3 ± 2.7
Longissimus pH at 24 hours	5.8 ± 0.2	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1
Carcass muscle score	3 ± 0	3 ± 0	3 ± 0	3 ± 0
NPPC Quality Scores				
Color	3 ± 0.3	3 ± 0.2	3 ± 0.1	3 ± 0
Marbling	3 ± 0.7	3 ± 0.8	3 ± 0.8	3 ± 0.9
Firmness	2 ± 0	2 ± 0	2 ± 0	2 ± 0
Hunter Color				
L*	55.54 ± 2.1	55.88 ± 2.4	56.40 ± 2.4	57.24 ± 2.4
a*	7.47 ± 0.9	7.58 ± 1.0	7.21 ± 1.0	7.17 ± 1.0
b*	13.88 ± 0.9	14.12 ± 0.9	13.88 ± 0.8	14.35 ± 0.6

Although some minor differences were observed in backfat thickness and meat color in clone progeny vs. comparators, none of these differences pose hazards that could affect food consumption risks. In addition, none of these differences would have any impact on the quality of the meat.

Table VI-32 provides the comparison of key nutrients between the progeny of clones and their comparators. Data were reported for 412 swine, of which 242 were the progeny of clones and 162 were the progeny of comparator boars. In total, 404 loin samples were analyzed, generating 23,432 data points. Of the 14,036 data points for offspring of clones, 29 values (0.2 percent) fell outside the respective range for controls. Twenty-eight of the samples corresponding to these values were thus re-assayed. (One sample was not re-assayed due to an oversight.) Following the second assay, only two data points exceeded the comparator range by more than 10 percent. One of these data points was for C18:1 octadecanoic (oleic) acid, a monounsaturated omega-9 fatty acid found in various animal and vegetable sources. The measured values for this parameter (5.44 percent and 5.66 percent) were well within the range for pork loin listed in the USDA National Nutrient Database (0.13 percent to 23.32 percent). In the sample that inadvertently omitted from re-assay, the value for threonine was 0.75 percent compared to 0.83-1.60 percent in the comparators, which also fell within the range listed in the USDA National Nutrient Database for pork (0.27 percent to 4.58 percent threonine). The only outlier in the entire data set was for C20:2 eicosadienoic acid. Concentrations of this analyte in a single sample were 0.04 percent and 0.06 percent, compared to 0.01 percent-0.03 percent in the comparators. No reference range was found for C20:2 eicosadienoic acid in pork. These results clearly demonstrate, that based on a large number of samples and composition-specific analytes that pork from the offspring of swine clones does not differ materially from pork from the offspring of conventionally bred swine (Walker et al., 2007).

Table VI-32: Comparison of Nutrient Concentrations in Meat from Progeny of Swine Clones and Comparators
(from ViaGen dataset, Appendix F, and Walker et al. 2007)

Nutrients[1]	Progeny from Clone Boars mean ± std. dev.	Progeny from Comparators Boars Mean ± std. dev.
Amino Acids		
Aspartic acid	2.31±0.19	2.29±0.16
Cystine	0.25±0.02	0.25±0.01
Glutamic acid	3.76±0.34	3.71±0.27
Glycine	1.14±0.15	1.12±0.13
Histidine	0.98±0.09	0.98±0.07
Isoleucine	1.03±0.12	1.03±0.10
Leucine	1.90±0.14	1.89±0.12
Lysine	2.06±0.17	2.07+0.16
Methionine	0.61±0.05	0.62±0.04
Phenylalanine	0.96±0.09	0.94±0.08
Praline	1.09±0.13	1.11±0.13
Serine	0.96±0.08	0.95±0.07
Threonine	1.09±0.09	1.08±0.07
Tyrosine	0.81±0.06	0.81±0.05
Valine	1.09±0.12	1.10±0.10
Fatty Acids and Cholesterol		
8:0 (Caprylic acid)	<0.01[2]	0.01
10:0 (Capric acid)	0.01±0.002	0.01±0.002
11:0	<0.01	<0.01
12:0 (Lauric acid)	0.01±0	0.01±0
14:0 (Myristic acid)	0.08±0.027	0.08 ± 0.029
14:1 (Myristoleic acid)	<0.01	<0.01
15:0	<0.01	<0.01
15:1	<0.01	<0.01
16:0 (Palmitic acid)	1.39±0.38	1.40±0.49
16:1 (Palmitoleic acid)	0.17±0.06	0.16±0.05
17:0 (Margaric acid)	0.01±0.003	0.01 ±0.002
17:1 (Margaroleic acid)	0.01±0.003	0.01±0.002
18:0 (Stearic acid)	0.66±0.24	0.68±0.25
18:1 (Oleic acid)	2.26±0.76	2.20±0.72
18:2 (Linoleic acid)	0.3±0.11	0.29±0.11
18:3 (Linolenic acid)	0.02±0.001	0.01±0.005
18:4	0.01±0.0001	0.01±0.004

Table VI-32: Comparison of Nutrient Concentrations in Meat from Progeny of Swine Clones and Comparators
(from ViaGen dataset, Appendix F, and Walker et al. 2007)

20:0 (Arachidic acid)	0.01±0.005	0.01±0.005
20:1 (Gadoleic acid)	0.08±0.04	0.07±0.04
20:2 (Eicosadienoic acid)	0.02±0.01	0.02±0.005
20:3 (Eicosatrienoic acid)	0.01±0.01	<0.01
20:4 (Arachidonic acid)	0.01±0.003	0.01±0
20:5 (Eicosapentaenoic acid)	0.01 ± 0	0.01±0.004
21:5 (Heneicosapentaenoic acid)	0.01±0	<0.01
22:0 (Behenic acid)	<0.01	<0.01
22:1 (Erucic acid)	0.01±0.006	0.02±0.006
22:2 (Docosadienoic acid)	<0.01	0.01±0.01
22:3 (Docosatrienoic acid)	<0.01	<0.01
22:4 (Docosatetraenoic acid)	<0.01	<0.01
22:5 (Docosapentaenoic acid)	<0.01	<0.01
22:6 (Docosahexaenoic acid)	0.02±0.01	0.02±0.01
24:0 (Lignoceric acid)	<0.01	<0.01
24:1 (Nervonic acid)	<0.01	<0.01
Cholesterol (mg/100 g)	57.93±5.46	59.39±5.04
Minerals		
Calcium	0.01±0.003	0.01±0.002
Iron	0.00±0.0005	0.000±0.003
Phosphorus	0.18±0.082	0.16±0.082
Zinc	0.00±0.0003	0.00±0.0001
Vitamins		
Niacin (mg/100g)	10.68±1.23	10.64±1.03
Vitamin B_6 (mg/100 g)	0.40±0.07	0.38±0.07
Vitamin B_{12} (mcg/100 g)	1.01±0.25	0.97±0.28

[1] Unless otherwise specified, quantities are expressed as g/100g homogenized meat.
[2] Values marked with "<" indicate concentrations below the level of detection for the instrument used in the assay.

In a study of the Jin Hua breed, Shibata et al. (2006) described carcass traits and meat quality of the offspring of cloned females. Sires were sexually produced (non-clone) Jin Hua boars. Forty-four offspring (23 male and 21 female) obtained from six litters produced by six dams were slaughtered at 70 kg body weight. Compared to non-cloned Jin Hua controls, offspring of cloned dams had shorter back and loin length (54.1 ± 0.3 cm vs. 56.0 ± .3 cm in controls), lower weight ratio of loin bacon (40.6 ± 0.2 percent vs. 41.9 ± 0.4 percent in controls) and higher weight ratio of ham (27.9 ± 0.3 percent vs. 26.5 ± 0.2 percent in controls). There were no differences between offspring of clones and control pigs in back fat thickness or loin area. Analysis of meat quality

indicated that meat from offspring of clones had a lower pH (5.47 ± 0.01 vs. 5.67 ± 0.03 in controls) and greater cooking loss (26.8 ± 0.3 percent vs. 24.9 ± 0.4 percent in controls). Water content, fat content, weep loss and shear value of meat from offspring of clones were not different from control values. The authors concluded that most of the meat quality characteristics of the Jin Hua breed were retained in the progeny of Jin Hua dam clones.

iii. Conclusions from Studies Evaluating the Composition of Meat and Milk from Clones and Their Progeny

The second prong of our Risk Assessment is based on the hypothesis that food products from healthy animal clones and their progeny that are not materially different from corresponding products from conventional animals are as safe to eat as their conventional counterparts. CVM has reviewed several peer-reviewed publications that have evaluated gross (e.g., milk yield, carcass characteristics) and fine (e.g., individual amino acid and fatty acid components) characteristics of meat and/milk from clones, and in two studies, their sexually-reproduced progeny. All but one of these studies indicate that none of the characteristics that we examined differed in any biologically significant way between the clone and comparator. The only exception is a preliminary study in bovine clones which provides evidence that lipid metabolism may be altered in clones, resulting in slight alterations in the fatty acid composition of milk and meat. However, without a comparison of these data to historical reference values, it is unclear whether these differences are representative of all bovine clones or are specific to the limited number of genotypes used in the study.

For swine clone progeny, a comprehensive, peer-reviewed analysis of meat from a large number of animals provides strong evidence that there are no compositional differences between meat from swine clones and meat from conventional swine, and that meat from clone progeny and their comparators is not materially different.

Therefore, in this prong of the Risk Assessment, CVM concludes that the weight of evidence indicates that meat and milk from clones and their progeny do not differ materially from meat and milk derived from their conventional counterparts, and therefore, based on compositional analysis, do not pose any additional food consumption risks compared with meat and milk from conventionally bred animals.

iv. Allergenicity and Feeding Studies in Rodents

The Report of the Japanese Research Institute for Animal Science in Biochemistry and Toxicology

In addition to data on composition of milk and meat from clones, the report of the Japanese Research Institute for Animal Science in Biochemistry and Toxicology (Japan 2002) included the results of studies on the allergenic potential of milk and meat derived from cattle clones as well as rodent feeding studies conducted with these products.

The ability to digest a protein is one index of potential allergenicity; a protein that is less digestible may be more likely to provide an allergenic response. The protein digestion rate of freeze-dried milk combined in feed consumed by rats is reported below for milk obtained from ordinary cattle, blastomere nuclear transfer (BNT) clone cattle, and SCNT clone cattle. The authors report that there was no biological difference among the groups tested.

Table VI-33: Protein Digestion of Freeze Dried Milk from BNT and SCNT Clones and "Ordinary" Cattle
(from Japan 2002)

Test Group	Number of Animals	Digestion Rate (mean ± standard deviation)
Ordinary Cattle	5	83.0 ± 2.6
BNT clone cattle	5	82.7 ± 2.0
SCNT clone cattle	5	8.13 ± 3.4

In a separate study, mice were sensitized by intraperitoneal injection to extracts of milk from clone and non-clone cows. Fourteen days later, the abdominal wall of the mice was surgically retracted and an allergic reaction induced by re-injection of the freeze-dried milk extract into the abdominal wall. Control mice did not receive the second injection of milk extract. Allergenic response was assessed based on vascular permeability as measured by the diameter of dye leakage from the site of injection. No statistically significant differences in allergenic activity were reported between groups. The data are presented in Table VI-34.

Table VI-34: Allergenic Response to Milk from BNT and SCNT Clones and Ordinary Cattle
(from Japan 2002)

Test Group	Mouse Group	Number of Animals	Diameter of dye leakage (mm) (mean ± standard deviation)
Ordinary Cattle	Control Group Test Group	7 10	7.0 ± 3.7 18.0 ± 2.9
BNT clone cattle	Control Group Test Group	7 10	4.7 ± 3.2 18.0 ± 3.9
SCNT clone cattle	Control Group Test Group	7 10	4.9 ± 4.6 17.9 ± 4.2

Based on these two studies, the authors conclude that there were no biologically or statistically significant differences in the allergenic potential of milk from ordinary cattle or BNT or SCNT clones.

To address possible allergenic potential of meat from clone cows, the Japanese researchers compared protein digestion rates with artificial digestive juice and in a rat model, and looked for an allergenic response following direct challenge in rats. For the *in vitro* digestion test, samples were taken from a one-day old conventional calf and a four day old clone. The rates of digestion by artificial digestive juices (artificial gastric juice and artificial intestinal juice) were compared for freeze dried meat derived from ordinary cattle, BNT clone cattle, or SCNT clone cattle (Table VI-35). No information is provided in the translation regarding the artificial digestive material. The results are presented below as the rate of protein digestion.

Table VI-35: Rates of *in vitro* Digestion of Beef from SCNT Clones or Ordinary Cattle
(from Japan 2002)

Digestive juice	Sample	Rate of digestion after the start of incubation (per cent)					
	Course	Start	0.75 hr	1.5 hr	3 hr	6 hr	12 hr
Artificial gastric juice	Ordinary beef	0	68	79	-	95	90
	Somatic cloned beef	0	59	78	-	91	90
Artificial intestinal juice	Ordinary beef	0	-	20	40	66	67
	Somatic cloned beef	0	-	28	38	67	63

It was concluded that there were no biologically significant differences in the rates of digestion for meat from ordinary beef cattle or from clone beef cattle using artificial digestive juices.

The protein digestion rate of freeze-dried meat combined in feed consumed by rats is in Table VI-36. The authors report that there was no biological difference among the groups tested.

Table VI-36: Protein digestion rate in rats following consumption of freeze dried meat from clone cattle and non-clone cattle
(from Japan 2002)

Test Group	Number of Animals	Digestion Rate (mean±s.d.)
Ordinary cattle beef	5	83.8 ± 6.6
BNT clones	5	82.3 ± 4.7
SCNT clones	5	84.9 ± 3.6

In a separate study, mice were given sensitizing intraperitoneal injections of extracts of freeze-dried beef from clone and non-clone cows. Fourteen days later, the abdominal wall of the mice was surgically exposed and an allergic reaction induced by re-injection of the freeze-dried beef extract into the abdominal wall and administered a vascular dye. Control mice did not receive the second injection of beef extract and only were administered the dye. Allergenic response was assessed based on vascular permeability as measured by the diameter of dye leakage. No statistically significant difference in allergenic activity was reported between groups. The data are presented in Table VI-37.

Table VI-37: Allergenic response by mice to intraperitoneal injection of extracts of freeze-dried beef 80from BNT and SCNT cloned cattle and ordinary cattle
(from Japan 2002)

Test Group	Mouse Group	Number of Animals	Diameter of dye leakage (mm) (mean±s.d.)
Ordinary cattle	Control group Test group	7 10	5.3 ± 5.0 13.0 ± 5.9
BNT clones	Control group Test group	7 10	7.0 ± 4.9 12.5 ± 3.5
SCNT clones	Control group Test group	7 10	5.7 ± 4.2 13.1 ± 5.0

The authors conclude that there were no biologically or statistically significant differences in the allergenic potential of meat from ordinary cattle or BNT or SCNT clone cattle.

The potential for milk and meat from BNT and SCNT clone cattle to cause clastogenic[109] (DNA breaking) events was assessed using an *in vivo* mouse micronucleus assay. Mice were fed milk or freeze dried powdered beef from ordinary cattle, BNT clone cattle, or SCNT clone cattle at 0, 2.5, 5, or 10 percent of the diet for 14 days. In addition, a positive control group received a single intraperitoneal injection of 2 mg/kg mitomycin C, a known clastogen. The positive control group showed a statistically significant increase in the incidence of micronuclei appearance and polychromatic erythrocyte rate, and was considered a positive test. No milk- or meat-fed group, whether derived from ordinary or clone cattle, caused mutations in this assay (i.e., no group fed beef derived from ordinary cattle or clone cattle was positive in this assay for mutagenicity or clastogenicity). The report concludes that there were no biologically significant differences in the component analysis or the results of feeding milk or meat from ordinary cattle, BNT clones, or SCNT clones.

[109] Clastogens are often referred to as mutagens, as most DNA breaks result in mutations if they do not first kill the cell or organism. The mouse micronucleus test examines the ability of a substance to cause the chromosomes of precursors of red blood cells in mice to break. Because mammalian red blood cells lose their nuclei as they mature, if any DNA is left in the mature blood cells, it is due to pieces of chromosomes breaking away from the rest of the nucleus as it is extruded from the immature blood cell during its maturation.

In a 28-day dose-range finding study, rats were fed diets containing freeze dried milk or beef from clones and ordinary cattle at concentrations of 0, 5, 10, or 20 percent of the diet.[110] General signs, body weight, food consumption, urinalysis, hematology, blood chemistry, and organ weights were compared between groups. English-language summary tables were provided in the original Japanese-language report and have been provided in Appendix I. No biologically significant differences were reported in rats fed milk or meat from clones compared to rats fed milk or meat from ordinary cattle. In addition, the report notes that 10 cattle fed clone milk powder at 2.5, 5, or 10 percent of the diet showed no significant differences in body weight increase, indicating that the milk did not contain anti-nutrients or other toxicants to cattle. The duration of exposure is not reported.

Finally, the report provides some data from a 14-week oral feeding study conducted in rats to determine the effects of a diet containing milk and meat derived from clone cattle. Results of this study were subsequently published (Yamaguchi et al., 2007) and are discussed in detail in the next section.

v. Peer-reviewed publications

In a review of the nutritional value of milk and meat from cattle clones, Tome et al. (2004) described a study in which Wistar rats were fed either a milk-based or meat-based diet for 3 weeks. Milk and meat were obtained from cloned animals at INRA or non-cloned control cattle. Effects on food intake, body weight gain, organ weights and fasting glucose levels were measured. No differences were observed in the response of rats receiving either meat or milk from clones compared to rats fed meat or milk from control cattle. In addition, no differences were detected for IgG, IgA or IgM subtypes for rats receiving clone- or non-clone-derived diets. Further, no specific anti-milk or meat protein IgE responses were detected in rat sera. These results provide preliminary evidence that the nutritional value of milk and meat derived from clones is not different from that of milk and meat from non-clone animals.

In 2007, Yamaguchi et al. reported the results of a long-term (14-week) feeding trial in which rats were fed various amounts of milk and meat derived from conventionally bred cattle and cattle derived from either embryonic or somatic cloning. The authors claim that this published report is the first to use standard toxicological methods to study the effects of feeding meat and

[110] Animal feeding studies to examine the toxicity of specific components of materials contained in foods are significant elements of a toxicity assessment. It is, however, generally recognized that animal feeding studies to examine the toxicological effects of whole foods (*i.e.*, feeding the whole food from a clone to the toxicology test animal) are of limited value due to the complex nature of the whole food, inability to provide sufficiently high doses of minor components of the whole food, and limited sensitivity of the assay. (Kessler et al. 1992; Codex Alimentarius, 2003 at ftp://ftp.fao.org/codex/Publications/Booklets/Biotech/Biotech_2003e.pdf).

milk from clones. Male and female rats (n=10 per group), approximately five weeks of age, were fed diets containing 1, 2.5 or 5 percent meat powder or 2.5, 5 or 10 percent milk powder derived from Japanese Black clones, Holstein clones, or non-cloned control cattle of the same breeds. Control rats were fed the basal diet, and diets containing meat or milk powder were formulated to be equal in nutritional value to the basal diet. Doses of meat and milk powder were based on a preliminary trial in which feeding 10 percent or 20 percent meat powder or 20 percent milk powder resulted in decreased feed intake, decreased body weight gain and (undescribed) clinical and pathological findings, or the equivalent of a maximum tolerated dose (MTD), the standard starting point for setting dosing for longer term toxicological studies. The authors speculate that adverse effects of feeding high concentrations (10-20 percent) of meat or milk were due to the nutritional imbalance of the diet (see footnote 28 for a discussion on why whole food feeding studies are not recommended by the US FDA). In addition to standard toxicological endpoints (e.g., clinical signs, body weight gain, feed intake, analysis of blood and urine), rats were subjected to a functional observational battery including reflex and sensory functions and locomotor activity.

None of the animals in the study died during its course. No abnormal clinical signs or changes in body weight or feed intake associated with consumption of meat or milk from either embryonic or SCNT clones. Body weight gain decreased was observed in one male rat exposed to the low dose of milk powder. Subsequent investigation of this rat indicated that it suffered from malocclusion (abnormal position of the teeth resulting in difficulty with chewing) leading the authors to conclude that the decreased weight gain was unrelated to exposure to milk powder from the SCNT clones. Average growth rate in rats fed milk or meat from embryonic or somatic clones, as indicated by body weight change, was not different from that in controls. Estrous cyclicity, assessed by vaginal smears on days 43-57 of the study, also was not affected by feeding milk or meat from either group of cattle clones.

The authors state that results of urinalyses conducted at weeks 4, 8, and 12 were similar between control rats and rats fed milk or meat from cattle clones. Similarly, the authors report no significant differences in hematology or blood chemistry between control and treated groups.

Compared to control animals, there were no differences found at necropsy in gross pathology or organ weights in rats fed milk or meat from cattle clones. Extensive histological findings are shown for 13 or 14 organs in rats fed meat or milk, respectively (testicular histology is missing from the group fed meat). Although numerous abnormal histological findings are reported, consumption of milk or meat from either embryonic or somatic clones did not increase the

incidence or severity of pathologies in any organ. The authors therefore concluded that the histological findings represent naturally occurring events unrelated to treatment.

Frequency of vocalization was included as part of the functional observational battery. At weeks 4, 8, and 12, changes were observed in the frequency of vocalizations between controls and rats fed milk or meat cattle clones. After comparing data from weeks 4, 8 and 12 with data from other weeks (data not shown), however, these frequencies were not consistently higher or lower than controls and, the authors concluded that the changes were incidental and unrelated to treatment. The authors reported that there was no effect of feeding milk or meat from either embryonic or SCNT clones on reflex, sensory functions, or locomotor activity.

The overall conclusion from this study is that consumption of meat or milk from cloned cattle did not alter the physiological condition of the rats. The study provides no evidence for a specific risk of consuming meat or milk from cattle derived from SCNT, which are not currently consumed by humans, compared to meat or milk from clones derived from embryonic cloning technology, which have been present in the Japanese food supply for several years.

Conclusions from Allergenicity and Feeding Studies in Rodents

The second prong of this risk assessment is based on the hypothesis that edible products from healthy cloned animals and their progeny are as safe to eat as edible products from conventionally produced livestock. CVM reviewed three studies in which the rat was used as a surrogate animal model to investigate possible biological effects of eating meat or milk from cattle clones. One of these feeding studies was conducted over an extended period (14 weeks) and included standard toxicological endpoints as well as a functional observational (behavior) battery. None of these studies demonstrated any change in the physiology or pathology of the rat following consumption of meat or milk from clones. Moreover, no evidence has been found to indicate that the allergenic potential of meat or milk from cloned cattle is greater than that of meat or milk from non-cloned cattle. No behavioral changes were observed. These findings are consistent with our conclusions using the Compositional Analysis approach, *i.e.,* that meat and milk from clones and their progeny are not materially different from meat and milk derived from conventional counterparts and thus do not pose any additional food consumption risks relative to food from conventional animals.

B. Drawing Conclusions Regarding Risks Associated with Consumption of Food Products from Animal Clones

1. Approaches for Decreasing Uncertainties

The fundamental problem in determining the quantity and types of data required to reduce the uncertainties associated with a judgment of "no additional risk" has bedeviled the scientific, risk, and regulatory communities. The impracticality of proving a negative and, in the absence of its proof, determining the consequent activities to identify the conditions under which concerns have been minimized to levels considered "acceptable" becomes the goal of a comprehensive risk assessment/management process.

In fact, certainty of prediction is unattainable in science. In its absence, risk assessment can provide risk managers with a systematic approach for bounding the "risk space" in which to operate by allowing assumptions and uncertainties to be clearly identified. Especially for new technologies in which uncertainty may be high, the "bounded framework" risk assessment process allows decision makers (both risk assessors and risk managers) to draw conclusions based on the data. Then, by explicitly addressing uncertainties, identifying biases, scientifically defensible (or alternatively, policy-based) judgments can be made about acceptable risk levels. The added benefit of such a process is that interested individuals are provided with a level of transparency that allows them to judge the quality of the science and the relative merits of decisions stemming from its evaluation.

This risk assessment has provided an overview of the molecular evidence for epigenetic dysregulation as the basis for obvious and subtle hazards that may arise in animal clones, the biological reasons for why subtle changes would not persist in progeny of healthy clones, the existing data on the health of animal clones and their progeny, and information on the composition of foods derived from clones and their progeny. These data can be incorporated into four procedural steps leading about to conclusions regarding food safety:

- *Bounding the risk space*, in which the "risk hypotheses" are explicitly identified and thereby the biases that influence the weight of the evidence evaluations regarding the health of the animals and the composition of food products derived from them;

- *Performing a weight of evidence evaluation of the data to characterize the risks* contained within the risk space, in which the information on food consumption hazards posed by cloning is summarized, and drawing conclusions based on the risk hypotheses presented in Step 1;

- *Characterizing the uncertainties* associated with the data and their interpretation, including identifying important data gaps based on Critical Biological Systems and Compositional Analysis approaches; and

- *In subsequent versions of this Risk Assessment, reevaluating previously estimated risks* based on new information to make new weight of evidence determinations.

2. Bounding the Risk Space

The two underlying risk hypotheses that explicitly bound the "risk space" in which the evaluations are being made are

- **Animal Clone Risk Hypothesis 1: *Clones are the Same as Sexually-Derived Animals***
  Animal clones *are* biological copies of the donor animal, and *data confirming* overall findings of animal health and food product comparability *are sufficient* to indicate that no additional risk is posed by the consumption of such food products.

- **Animal Clone Risk Hypothesis 2: *Clones are Different from Sexually-Derived Animals***
  Animal clones *may appear to be* faithful biological copies of the donor animal, but subtle hazards may have resulted from incomplete or inappropriate reprogramming of the genome as part of the SCNT process. In order to avoid additional risks above those posed by consumption of foods from sexually-derived animals under this hypothesis, *comprehensive health and compositional data* must be collected and analyzed to demonstrate that the animals are healthy, and that food products derived from them do not differ significantly from sexually-derived animals.

- **Clone Progeny Risk Hypothesis: *Gametogenesis Resets Epigenetic Dysregulation***
  Normal, healthy clones reproducing via sexual reproduction give rise to progeny animals that are as healthy as animals derived from any other sexual reproduction event.

3. Developing Conclusions Regarding Food Consumption Risks

The conclusions that can be drawn with respect to the safety of consuming food products from animal clones and their progeny based on the data reviewed in this Risk Assessment follow. Because risk assessment is best performed recursively, risk assessment conclusions should always be considered to apply to the dataset that was examined; each conclusion is based on the

information that was available for consideration, but if additional data become available, a conclusion may change, or the degree of confidence placed in the conclusion may be adjusted. Nonetheless, risk managers need to make decisions at particular points in time, and despite the desire for recursive assessments, decisions often include statements about the degree of certainty that accompany them.

Each conclusion is followed by a statement on whether the judgment comes from application of Hypothesis 1 (Assumes Clones are the same as Sexually-Derived Animals), or Hypothesis 2 (Assumes Clones are Different from Sexually-Derived Animals), and the reason for the selection of that hypothesis (and its implicit bias).

As previously stated, the Risk Assessment assumes that all of the laws and regulations that apply to sexually-derived animals and the food products that come from them apply equally to animal clones, their progeny, and food products that are derived from them.

Our weight of evidence risk assessment conclusions are presented on a species-specific basis, except for bovine clones, where the large dataset allows for the consideration of individual developmental nodes. The weight of evidence evaluations take into account:

- All of the observations for that species (or developmental node);
- The extent to which those observations are coherent with biological assumptions;
- The consistency with which those observations are also seen across species, including the mouse model, where applicable;
- Uncertainties that persist in the evaluation, including the source of those uncertainties; and
- The confidence level in the conclusion based on all of the preceding considerations.

Because this is a qualitative, comparative risk assessment, it does not attempt to assign quantitative values to estimates of risk or safety. The strongest conclusions that can be drawn regarding positive outcomes in risk assessments of this type are “no additional risk” because outcomes are weighed against known comparators. In the context of edible products derived from clones, a finding of no additional risk means that food products derived from animal clones will not pose any additional risks relative to corresponding products from non-clones, or are as safe as foods we eat every day. As with all risk assessments, some uncertainty is inherent either in the approach we have used or in the data themselves. For each conclusion, CVM has attempted to identify the sources and extent of these uncertainties. A more complete discussion of sources of uncertainties and their implications can be found in Chapter VII.

4. Weight of Evidence Conclusions Regarding Food Consumption Risks for Clones and their Progeny

Based on this review of the body of data on the health of animal clones, the composition of meat and milk from those animals and corresponding information on clone progeny, CVM has drawn the following conclusions:

a. Cattle Clones

Edible products from perinatal bovine clones may pose some very limited human food consumption risk.

The underlying biological assumption in place for this age cohort is that perinatal clones may be physiologically more unstable at birth due to residual incomplete or inappropriate reprogramming of the donor nucleus. Data from both the peer-reviewed publications and Cyagra are consistent with that assumption; some perinatal clones do not survive for several reasons, including poor placentation, LOS, and in some cases, frank malformations. Although the health of surviving clones can be unstable for a period of time, many survivors tend to adjust to life outside the womb within a relatively short period, either on their own or with assistance from caregivers (see Juvenile Developmental Node). The peer-reviewed literature and Cyagra data indicate that, depending on the laboratory, a significant proportion of perinatal clones survive gestation and are born without significant health problems. Laboratory measures of key physiological functions do not appear to indicate that surviving animals are very different from conventional newborns. It is therefore unlikely that food consumption risks have been introduced into these animals.

The uncertainty associated with the preceding statement is relatively high, however, for the following reasons. First, postulated differences in epigenetic reprogramming between perinatal clones and comparators suggest that some subtle hazards may have been introduced into these animals. Second, the relatively poor condition of many of these perinatal clones also precludes the conclusion that no food consumption risks, such as nutritional imbalances, are present. Therefore, given that perinatal clones may differ from comparator animals of the same age, at this time, the Center concludes that they may pose a very limited nutritional risk for consumption as food.

i. Risk Hypothesis Statement for Perinatal Bovine Clones

At this time there is insufficient information to move from Hypothesis 2 (Clones are Different) to Hypothesis 1 (Clones are the Same), even though the available data neither identify nor predict

the presence of food consumption hazards (and subsequent risks) from these very young clones. The uncertainties in the data are relatively high and lead the Center to have a relatively low degree of confidence in the safety of edible products from perinatal bovine clones. We note, however, that it is highly unlikely that clones of this age group would be consumed for food.

A possible disposal method for clone calves that die or are euthanized due to poor health during the perinatal period is rendering.[111] Rendered materials have many uses, including ingredients in animal feed (meat and bone meal). Thus, it is important to consider possible risks to food animals that consume rendered materials derived from clones, as well as the possible risk to humans who consume those food animals. Although the Center has concluded that perinatal clone calves may pose some limited risk human consumption, we do not believe that the nature of this risk is unique to cloned calves because all of the pathologies observed in these calves are also observed (at lower rates) in calves produced using other ART methods. Therefore, there is no *a priori* reason to suggest that products derived from rendering perinatal clone calves would have any unique properties compared to rendered products derived from non-clone calves. The Center concludes that rendering perinatal clone calves will not pose risks, either to animals (via animal feed) or to humans consuming animals fed rendered material derived from clone cattle.

Edible products from juvenile bovine clones pose no additional food consumption risks relative to corresponding products from contemporary conventional comparators.

The underlying biological assumption for this developmental node is that if any anomalies were found in the youngest clones and those animals survived to be healthy adults, the juvenile developmental node would be a period of equilibration and normalization. The data appear to be consistent with such a hypothesis.

Clone calves that survive the perinatal period and are not affected by congenital abnormalities appear healthy and demonstrate normal patterns of growth and development. None of the physiological measures taken, including both clinical chemistry and hematology, indicated any food consumption hazards in these animals. For some bovine clones, the health problems observed during the perinatal period appears to extend into the juvenile period, resulting in an increased risk of morbidity and mortality in calf clones during the first six months of life. This risk appears to be the result of sequellae of developmental abnormalities present at birth that persist beyond the perinatal period (*e.g.,* musculoskeletal defects, prolonged recumbency, enlarged umbilicus, respiratory distress, poor thermoregulation). However, morbid animals

[111] Rendering is a process that uses heat (cooking), extraction of moisture, and separation of fat to convert animal tissues into stable, value-added materials. Material suitable for, and commonly used in rendering includes inedible tissues from slaughtered animals, carcasses that are condemned upon inspection, and sick animals that die on farm or in transit. The high temperatures used in the rendering process effectively destroy foodborne pathogenic microorganisms (Meeker 2006).

bearing these problems are not expected to contribute to food consumption risks because they are not expected to pass the antemortem inspection required to enter the food supply. No new or additional health risks were identified during the juvenile period other than those observed during the perinatal node. Therefore, the Center concludes that healthy juvenile bovine clones are not different from comparator animals of the same age, and pose no additional food consumption risk.

ii. Risk Hypothesis Statement for Juvenile Bovine Clones

The assessment began at the position of Hypothesis 2, but the scientific evidence has moved the assessment from Hypothesis 2 to Hypothesis 1 for maturing juvenile clones. The weight of the evidence and the underlying biological assumptions lead the Center to conclude that there would not likely be any additional risk from the consumption of food from healthy juvenile clones relative to corresponding products from their conventional comparators. The consistency of these observations across all of the data for juvenile bovine clones makes the uncertainty associated with this judgment relatively low, and provides the Center with a relatively high degree of confidence in judgments regarding the health of (and consequent food safety of edible products derived from) this age cohort of bovine clones.

Edible products derived from adult bovine clones pose no additional risk(s) relative to corresponding products from contemporary conventional comparators. This conclusion is based on application of both prongs (CBSA and Compositional Analysis) of the risk assessment approach.

The body of data comprising the CBSA approach on adult domestic livestock clones is made up of two components: data and information extracted from peer-reviewed publications and the Cyagra dataset. The empirical evidence on the health of these animals is consistent with the biological prediction that there are no underlying biological reasons to suspect that healthy animal clones pose more of a food safety concern than conventional animals of similar age and species.

The data from Cyagra survey indicate that healthy clones of the oldest cohort (6-18 months) are virtually indistinguishable from their comparators even at the level of clinical chemistry and hematology. These data also confirm the observation that physiological instabilities noted earlier in the lives of the clones are resolved juvenile developmental node (see previous conclusions regarding other developmental nodes), and do not reappear as the clones age. The statements regarding the health and apparent normality of animals of this age group from the peer-reviewed literature are consistent with the data evaluated by CVM. There are some reports of early deaths of clones; as these animals would not enter the food supply, they do not

pose a food consumption risk. Data on reproductive function in cows or bulls of this age cohort indicates that that healthy bovine clones surviving to reproductive maturity function normally and produce healthy offspring. These data are consistent across studies. Given that reproduction is the most difficult "biological hurdle" placed on an organism, the observation of normal reproductive function provides an additional degree of confidence to the conclusion of the appropriate development of these animals.

The reports on the composition analysis of meat or milk from bovine clones do not indicate that there are biologically significant differences in the composition of meat milk derived from clones and conventionally bred cattle. Although these comparisons are important for determining the effects of cloning on tissue composition (and therefore food consumption risks), it is equally, if not more important to consider historical or reference values. None of the observed values that could be associated with cloning fell outside historical reference ranges, and were also observed in meat or milk from conventionally bred animals. These observations illustrate the wide variability in the composition of milk and meat in general, and indicate that cattle clones produce meat and milk that display similar ranges of variability.

No novel components have been identified in meat or milk from clone cattle, and data from one report show no difference in allergenic potential for meat or milk derived from cattle clones compared with meat or milk from non-clone comparators. Similarly, neither meat nor milk from clone or non-clone cattle induced mutations in a mutagenicity assay (Japan 2004). Finally, none of the reports identified hazards, subtle or otherwise, that could pose food consumption risks.

iii. Risk Hypothesis Statement for "Adult" Bovine Clones

The assessment began at the position of Hypothesis 2: that animal clones may appear to be copies of the donor animal, but that the process of cloning may have introduced subtle hazards that could pose food consumption risks. As presented above, however, the weight of the evidence has moved the assessment from Hypothesis 2 to Hypothesis 1 (Clones are the same as their sexually-derived counterparts). Extensive and consistent empirical evidence, including epigenetic, physiological, and health data on individual animals and compositional analysis of milk and meat derived from individual animals, indicate that adult bovine clones are biologically equivalent to their contemporary comparators. Therefore, evidence confirming the health of the animals produced via similar methods, and evidence confirming the compositional similarity of meat and milk from clone and non-clone cattle indicates that there is no additional risk from the consumption of edible products from these animals relative to sexually-derived comparators. The consistency of the observations provide the Center with a high degree of confidence in judgments regarding the health of (and food safety of edible products derived from) this age cohort of bovine clones.

We note that given the economic considerations involved, it is not likely that many adult clones would enter the food supply as meat at this stage of the technology, unless they had suffered a non-treatable injury or old age. Milk products from lactating female bovine clones, however, could be introduced into the food supply.

b. Swine Clones

Edible products from adult swine clones pose no additional risk(s) relative to corresponding products from contemporary conventional comparators.

This conclusion is based on the same underlying biological assumption as cited for adult bovine clones (*i.e.,* non-transgenic clones would not likely express toxicants, no exogenous genes, and diseased animals would not be slaughtered for food). Because the data are more heavily weighted towards adult, market sized animals, judgments regarding the safety of food products from swine clones are provided in one aggregate set of comments.

Although generating swine clones appears to pose more technical difficulties than bovine clones, once piglets are born, the vast majority appear to be healthy. The health status of perinatal and juvenile animals is generally presented as "normal" or "healthy" in peer-reviewed publications. Results of one study conducted during the juvenile period indicate that the acute phase immune response may be altered in piglet clones. However, the overall implications of this study for the health of swine clones is unclear because the observations were made in only two piglets at a single time point in the life of the animals and the results have not been confirmed in other studies. Data from Archer et al. (2003a,b) and the ViaGen dataset do not indicate that older swine clones are more susceptible to infection. Therefore, even if the acute phase response of clone piglets is altered, it does not appear that this alteration affects their continued growth during the Juvenile Node or their health during the Reproductive or Adult Nodes. Further, data from Archer et al. (2003a,b) and the ViaGen dataset do not indicate that older swine clones are more susceptible to infection than their comparators.

The most compelling argument for the normal health status of swine clones has been presented by Archer et al. (2003a,b), who evaluated the behavior and physiological status of a small cohort of relatively young (15 weeks), and approximately market age (27 weeks) swine clones relative to closely related conventional pigs. Age-related physiological measures appeared to be normal, as demonstrated by levels of measures of growth such as alkaline phosphatase, calcium, and phosphorus and measures of immune system maturity such as globulin. No significant differences were observed in either behavior, epigenetic, or physiological measurements, indicating that these animals were not materially different from the comparators.

The data on the ViaGen clones (Appendix F) are on a relatively small number of animals, reared in very unusual settings (i.e., deprivation of colostrum, initial husbandry in pathogen-free conditions, switching to commercial settings) and are therefore confounded with respect to outcome. Nonetheless, the data indicate that even though the clone barrows were subjected to a significant immunological challenge after moving from pathogen-free conditions to more standard housing conditions, most clones were able to respond appropriately to this stress. Nonetheless, carcass qualitative characteristics were similar for clones and comparators in the ViaGen Dataset. Further, reproductive performance for these clone boars appears normal. No differences were noted in semen quality between clones and comparator boars; farrowing rates and litter sizes were within national averages. No biologically relevant differences were observed in the composition of meat from clones or comparators.

i. Risk Hypothesis Statement for Swine Clones

Based on both underlying biological assumption and confirmatory data, CVM concludes that consumption of food from healthy adult swine clones would not pose an additional risk above consumption of their conventional counterparts. The data from Archer et al. (2003a,b) is particularly compelling as it includes data on behavior, epigenetic reprogramming, and physiological measurements at two time points in the development of these clones. Likewise, data from ViaGen includes information on growth and reproduction, indicating that swine clones are not materially different from age-matched, genetically related swine. Taken together, these results support a Risk Hypothesis Level of 1 (clones are the same as sexually-derived animals) because they demonstrate a relatively high certainty based on biological plausibility, consistency of observations among different and compelling datasets, and consistency with responses observed across other clone species.

c. Sheep Clones

Except by relying on underlying biological assumptions, and by inference from other species, there is insufficient information on the health status of sheep clones to draw conclusions with respect to potential risks that could be posed from the consumption of food products.

With the exception of reports on Dolly, CVM was unable to find any publicly available reports on the health status of live sheep clones. There are several studies addressing methodological issues for optimizing the generation of clones, but these do not address post-natal health. There are reports of anomalies noted in fetal sheep clones that have died or been terminated, and reports on the pathology associated with animals that do not survive. Although these are instructive for understanding the molecular and developmental pathways that may be perturbed

during the process of SCNT, these studies have limited relevance to addressing food safety because the deceased animals would not have been allowed to enter the food supply. CVM was not able to find any reports on the composition of milk or meat from sheep clones.

i. Risk Hypothesis Statement for Sheep Clones

At this time there is insufficient information to support Hypothesis 1; Hypothesis 2 must be the default position with respect to potential food consumption risks from sheep clones. CVM was not able to find any studies providing specific evidence to show that sheep generated by SCNT are healthy and normal, and would therefore pose no additional food safety concerns beyond those of their conventional counterparts.

d. Goat Clones

Edible products from goat clones pose no additional food consumption risk(s) relative to corresponding products from contemporary conventional comparators.

This conclusion is based on the same underlying biological assumption cited for the other livestock species, and a relatively small but compelling dataset. Once clone embryos are transferred to surrogate dams and pregnancies are confirmed, the "success rate" for live births is quite high. The only anomaly noted was that approximately half of the cohort of goats reported on by Keefer et al. (2001a) appeared to have poor suckling response immediately after birth, but by the second day were responding normally and nursing from their surrogate dams. The animals appear to have developed well through reproductive age. The available data indicate their physiological responses are appropriate for age and breed. The reproductive development and function of male Nigerian Dwarf goat clones demonstrate that those animals functioned appropriately relative to age- and breed-matched comparators. One male progeny goat was derived from the buck clones; this animal also appeared to function in an age- and breed-appropriate manner. No meat or milk composition data were identified for goat clones.

i. Risk Hypothesis Statement for Goat Clones

Although the assessment began at Hypothesis 2, based on the underlying biological assumptions stated for the other clone species, consistency of responses with other species of clones, and a small but relatively rich dataset, CVM concludes that Hypothesis 1 more appropriately represents the conclusions regarding the food safety of goat clones. CVM places particularly high weight on the study of reproductive function, as it is one of the most complex physiological pathways to coordinate. The consistency of appropriate reproductive function, even in a small cohort of animals, adds to the confidence that can be placed in the judgment that these animals are as normal and healthy as their sexually-derived counterparts. Based on this finding, edible products from goats are not anticipated to pose more of a food consumption risk than their

sexually-derived counterparts. Further, given the data on the normal reproductive function of these animals, and a preliminary report of normal reproductive function of one male offspring of a male goat clone, CVM has more confidence in the empirical demonstration that clone progeny are as healthy as other sexually-derived animals.

e. Clone Progeny

Edible products derived from the progeny of clones pose no additional food consumption risk(s) relative to corresponding products from other animals.

Relative to the amount of meat and milk derived directly from clones in the U.S., it is likely that more food products (both meat and dairy) will be produced by the progeny of clones. Unlike their clone parents, progeny of clones are produced by sexual reproduction. The underlying biological assumption for health of progeny animals is that passage through the process of creating the cells that ultimately become ova and sperm naturally resets epigenetic signals for gene expression. This process is thought to effectively "clear" the genome of incomplete or inappropriate signals, and thus preclude genetic transmission of abnormalities from clones to their offspring. None of abnormalities that are observed in clone cattle have been reported in their progeny. For the progeny of swine clones, peer-reviewed studies together with extensive information in the ViaGen dataset provide direct data to indicate that these progeny are healthy at all developmental stages, and show that the composition of meat from the progeny of swine clones does not differ materially from pork derived from the offspring of conventionally bred swine. Both the cattle and swine data support the underlying biological assumption that the progeny of clone animals are essentially indistinguishable from the comparable progeny of other sexually derived animals.

ii. Risk Hypothesis Statement for Clone Progeny

Supported by extensive empirical evidence in the progeny of swine clones, we concur with the high degree of confidence that the outside scientific community (NAS 2002a,b) places in the underlying biological assumption that any abnormalities observed in clones will not be transmitted to their sexually-produced progeny. CVM therefore concludes that consumption of edible products from clone progeny will not pose any additional food consumption risk(s) relative to consumption of similar products from other sexually-derived animals.

5. Summary of Risk Hypotheses

The current weight of evidence suggests that there are no biological reasons, based on empirical data and underlying biological assumptions, to indicate that consumption of edible products from cattle, pigs, or goat clones poses a greater risk than consumption of those products from their non-clone counterparts. No frank or subtle hazards that could result in food consumption risks were identified in either the studies of the health of clones or in studies of the composition of milk or meat from clones. The level of certainty for these conclusions is highest for bovine clones, followed closely in degree of certainty by swine and, and then goat clones. The lack of species specific data for sheep clones precludes an evaluation of the risk for consumption of sheep clones at this time.

Consumption of edible products from the progeny of clones poses no additional risk(s) relative to those from other sexually-derived animals, based on underlying biological assumptions (supported by evidence from the mouse model system) and compelling empirical evidence on the health of clone progeny and their meat composition.

The level of confidence that may be placed in these overall conclusions is quite high, although additional data can always increase confidence.

a. Additional Issues

In addition to the hazards and risks described in the preceding portion of this risk assessment, there are a few issues that do not fit neatly into one of the categories that have been discussed previously. Many of these are overarching issues that may also have applicability to technologies other than SCNT.

i. Potential Allergenicity

The issue of allergenicity is one that is often cited for foods that do not have a long history of consumption. Although there is no reason to suspect that cloning will cause the synthesis of new proteins in animals that appear healthy and normal, there are two possible pathways that might pose an increased allergenic risk from the edible products of animal clones. One is an increase in the relative amount of an individual protein component of milk or meat that may only be present in very low or trace amounts. Cows' milk has been associated with true allergies (Cows Milk Allergy or CMA) in approximately six percent of the US population (Bernstein 2003). Caseins, although the predominant proteins in milk, do not appear to be the key allergens associated with CMA. The other possible pathway is that processing of the proteins during their generation in the mammary gland or muscle cells somehow alters their antigenic presentation. The Center cautions that these are purely hypothetical pathways, and that there has been no demonstration that either of these actually occurs.

In theory, evaluating the relative concentrations of milk proteins in clone and comparator milk could provide information to determine if the first risk exists. The study by Tian et al. (2005) provides just such a comparison using SDS/page technology. In practice, however, even this study highlights the difficulty in establishing the appropriate comparator and minimizing variability. Milk from non-clone dairy animals may vary in relative composition due to the influences of breed, diet, number of lactations, where in the lactation cycle the milk is collected, etc. Further, the level of exposure (dose) required to elicit an allergenic response is not well understood, and has been the subject of much discussion in the scientific literature (Taylor 2002) and among international regulatory bodies (Codex Alimentarius 2003[112]). Nonetheless, the limited studies provided (Japan 2002) show that milk from both SCNT and BNT clone cattle showed similar digestibility characteristics both *in vitro* and in a rodent *in vivo* assay. In addition, a rodent bioassay for allergic response did not show any significant differences in response between clone and non-clone derived milk. Combined with the underlying biological assumptions, these data support the lack of a unique allergic response to milk derived from clone cattle.

Similar risks are not likely to occur for meats, as meat allergies are so much less prevalent in the population that they are almost considered idiosyncratic, and individuals likely to suffer from meat-related allergies are likely to avoid those meats entirely. In addition, freeze dried meat from clone and non-clone cattle produced no difference in response in digestibility in both an in-vitro and rodent in-vivo assay, and there was no difference in difference in response in a rodent allergenicity bioassay (Japan 2002).

Finally, it is important to remember that relative and potential allergenicity in food is an issue that vexes the scientific and regulatory communities. FDA supports further research into the overall risk factors that cause individuals to exhibit aberrant immune responses. The agency has been actively involved in the evaluation of predictive tests at the laboratory and clinical level that address changes in protein structure and presentation. Nonetheless, it is important to remember that efforts such as those undertaken by the ILSI Allergy and Immunology Institute, the International Biotechnology Council, the National Academy of Sciences, Food and Agriculture Organization and the World Health Organization and the Codex Alimentarius address the allergenicity of *novel* proteins. These proteins are either new to the food supply as the result of the introduction of new foods, or are present in different matrices, as may be the case with transgenic plants or animals.

ii. Microbiological Effects

[112] ftp://ftp.fao.org/codex/Publications/Booklets/Biotech/Biotech_2003e.pdf

It has been suggested that epigenetic changes in animal clones could somehow alter the rumen and intestinal microflora of the ruminants (cattle, sheep, and goats), or the intestinal microflora of monogastric species (swine) (NAS 2002b). Such alterations in intestinal flora might be considered hazards because they could, in theory, result in increased levels of an existing zoönotic pathogen or the growth of a novel zoönotic pathogen. Shedding of these pathogens in fecal material could possibly result in a higher load of undesirable microbes on the carcass at slaughter, increasing the likelihood of contamination of the edible tissues.

The use of animal drugs has been postulated to alter the intestinal flora of treated food animals, resulting in an increased load of zoönotic pathogens in the food supply. The potential for animal drugs to induce this change was considered at length by the January 2002 CVM Veterinary Medical Advisory Committee on that topic[113]. Most of this independent scientific advisory committee found that animal drug use was unlikely to significantly impact pathogen load (or the prevalence of zoönotic pathogens), and that pathogen load has little or no impact on public health.

The Center is not aware of any studies that have characterized the intestinal flora of livestock clones, and the complexity of the intestinal microflora makes this an extremely difficult question to address directly. Although it is possible that epigenetic reprogramming in clones may have effects on the intestinal flora, this postulate can be challenged on the basis of animal health. The data reviewed in this risk assessment indicate that the vast majority of clones studied during the juvenile, reproductive and post-pubertal phases of life are as healthy as their sexually-produced counterparts. It therefore seems very unlikely that the milieu of intestinal microflora is abnormal in these animals, and that contamination of carcasses of clones due to bacterial shedding would pose a greater food consumption risk than that posed by conventional food animals.[114] We further note that such alterations would not be unique to clones as all animals, regardless of their method of production, are subject to alterations in epigenetic programming.

iii. Unanticipated Effects

This risk assessment has attempted to identify the range of potential hazards and risks that could be generated as the result of SCNT in domestic livestock species. Although it may be possible for a healthy clone to express some proteins inappropriately, the same argument can just as easily

[113] http://www.fda.gov/cvm/VMAC/winter2002meet.htm

[114] A possible exception to this conclusion may be cattle clones during the perinatal period, many of which have impaired health that could result in higher than normal fecal shedding of pathogens. However, it is unlikely that very sick calves or calves with internal abnormalities would enter the human food supply because they would not pass state or USDA inspection (as required by the Federal Meat Inspection Act; http://www.fsis.usda.gov/home/index.asp).

be made for sexually-derived animals. At this time, there is no validated method for determining small differences in protein constituents in foods, and even if such methodologies existed, the question would still remain as to how to interpret them--what foods would be used as comparators, and what degree of variability would be considered to pose a risk (NAS 2004)?

Finally, the issue of the hypothetical dysregulation of endogenous substances that may pose a hazard by virtue of increased dose should be addressed. The primary concern in this case is the up-regulation of small molecules that may retain bioactivity in the bodies of the human (or animal) food consumer, usually by virtue of the lack of degradation in the intestinal tract. For example, levels of endogenous substances that have posed some public concern in the past (*e.g.,* estrogen and IGF-I) have been evaluated in bovine clones, and based on those data, there is no reason to expect that the levels of these substances in clones would pose any food consumption risks for humans.

iv. Technology Changes

This risk assessment has focused on the outcomes of cloning (*i.e.,* clones and their progeny) rather than on the cloning process itself. As discussed in Chapter II and elsewhere, however, at the time this risk assessment was developed, most clone producers use the same overall technology to produce clones. Clearly, different producers and laboratories may modify the process to enhance the overall success rate of the cloning process. In general, however, the clones that were evaluated in this risk assessment were produced by very similar processes. From a risk perspective, the important constant in technology used to produce these clones is that donor nuclei and recipient oöcytes (or oöplasts) are not significantly manipulated beyond the obvious steps described in Chapter II. Thus, hazards other than epigenetic dysregulation are not introduced into clones.

Significant changes in cloning technology, especially those accompanied by donor nucleus or oöcyte treatment regimens introducing new hazards into the overall process, would significantly increase the uncertainty associated with our judgments regarding the degree of risk that could accompany the resulting clones and clone food products. Without a careful evaluation of the animals arising from such methods, it would not be appropriate to speculate on the relative safety of the process from either an animal health or food safety perspective.

6. How Much (Information) Is Enough?

The question of determining when sufficient data have been collected in order to allow high confidence in risk-based decisions regarding edible products from animal clones is difficult to

determine in the abstract. In practice, the answer is "it depends on what questions you ask, and how the data answer those questions."

Because the nature of the technology has generally precluded generating large datasets on clones with good statistical power, CVM constructed a systematic approach to frame the appropriate questions (hazard identification), evaluated the available data (hazard characterization), and attempted to characterize resulting risk (probability of harm given that exposure occurs). This weight of evidence approach allows for the evaluation of the data from the CBSA and Compositional Analysis prongs of the Risk Assessment as part of an overarching whole. The conclusions from this risk assessment represent the judgment of CVM veterinarians, animal scientists, toxicologists, and risk assessors. The underlying assumptions for clones and their progeny were that the animals needed to meet all relevant federal, state, and local laws and regulations for conventional animals, and the food products derived from clones or their progeny also had to meet relevant federal, state, and local laws and regulations.

When considered across the Developmental Node spectrum, the data on the health of livestock clones were remarkably consistent across species, despite initial anomalies that appear to be species-specific. For example, although LOS may be more prevalent in cattle and sheep, most surviving animals normalize initial anomalies and become "healthy and normal." This consistency has increased the value of even small datasets (*e.g.,* goats), and has contributed significantly to the judgments regarding the health of these clones and their suitability as food sources. In addition, CVM evaluated a number of reports on the composition of meat and milk from clones and their progeny. No biologically important or safety-relevant differences were noted when compositions were compared to standard databases or contemporary comparator controls. If anything, these data confirm the rather wide variability in the composition of meat and milk eaten on a daily basis. In summary, no toxicological hazard of concern for the human consumer has been identified in any of the reported studies. Although additional data from other sets of animals, particularly in other species routinely used for food, could be useful in increasing the confidence that may be placed in overall judgments regarding food safety, the weight of the evidence at this time is sufficient for the agency to draw the conclusions it has made in this Risk Assessment with reasonable certainty.

Chapter VII:

Summary and Conclusions

Chapter VII
Summary and Conclusions

Somatic Cell Nuclear Transfer (SCNT) is a technology still relatively early in its development. Cloning has been accomplished in relatively few species, with most of our current information stemming from studies in cattle, swine, goats, and mice. This Risk Assessment has addressed the hazards and potential risks that may be experienced by domestic livestock (*i.e.,* cattle, swine, sheep, and goats) involved in the cloning process (Animal Health Risks) and whether edible products from animal clones or their progeny pose food consumption risks beyond those of their conventional counterparts (Food Consumption Risks).

A. Methodology

This Risk Assessment used two complementary approaches, the *Critical Biological Systems Approach* (CBSA) and *Compositional Analysis Approach*, to identify and characterize potential animal health and food consumption hazards associated with cloning. It then employed a weight of evidence approach to draw conclusions regarding risks to animal health and for consumption of food products from clones and their progeny. This weight of evidence approach consisted of four steps:

(1)*Evaluation of the empirical evidence* (*i.e.,* data on molecular mechanisms, physiological measurements, veterinary records, and observations of general health and behavior) for the species being considered;

(2) *Consideration of biological assumptions* predicated on our growing understanding of the molecular mechanisms involved in mammalian development;

(3) *Evaluation of the coherence of the observations* with predictions based on biological mechanisms; and

(4) *Evaluation of the consistency of observations* across all of the species considered, including the mouse model system.

The Risk Assessment also assumed that animal clones, their progeny, and all food products derived from either clones or progeny must meet the same federal, state, and

local laws and regulations as food from conventionally bred animals.

Because no exogenous genes have been introduced into animals derived via SCNT, the underlying assumption has been that adverse outcomes observed in animal clones arise from epigenetic modifications due to incomplete reprogramming of the donor cell nucleus. Methodological and technological components (*e.g.,* selection of donor cell, cell cycle stage, *in vitro* factors associated with the SCNT process) may also affect outcomes as they do for other ARTs.

B. Conclusions Regarding Risks to Animal Health

To assess the health of animal clones for both the animal health and food consumption risk portions of this risk assessment, we used the *Critical Biological Systems Approach* (CBSA), which divides the life cycle of clones into five distinct Developmental Nodes. Available data for each species were sorted into these Developmental Nodes to evaluate the data systematically and to determine whether there are common developmental difficulties among the livestock clones or whether animals "recover" from initial infirmities related to cloning.

The results of the CBSA indicated that significant adverse health outcomes have been reported for cattle and sheep clones and their surrogate dams. These tend to include dystocia and high gestational mortality. In cattle and sheep clones, post-natal mortality tends to be concentrated in the perinatal period, and is higher in clones than in animals produced using other assisted reproductive technologies (ARTs).

To date, no adverse outcomes have been noted in clones that have not been observed in animals derived via other ARTs or natural mating. Goats and swine appear to develop without significant abnormalities. The incidence of adverse outcomes in cattle and sheep clones, however, appears to be higher than in other forms of ARTs. Common adverse developmental outcomes that have been observed in cattle and sheep generally fall under the heading of Large Offspring Syndrome (LOS), although there may be others. Newborn animals with LOS tend to be bigger than average for their breed and species, may show edema or other abnormalities of the lungs and other parts of the body, and exhibit cardiovascular and respiratory problems.

Most animal clones that survive the critical perinatal period appear to grow and develop normally. Even animals with physiological perturbations, including less severe manifestations of LOS, seem to resolve them, usually within a period of weeks. More severe complications of LOS may persist into the juvenile period, but clones do not

appear to develop any additional health risks unrelated to those that were observed during the perinatal period. Clones that reach reproductive age appear to be normal in all of the measures that have thus far been investigated, and appear to give rise to healthy, apparently normal progeny. Mature clones appear normal and healthy, and are virtually indistinguishable from their conventionally bred counterparts.

Studies that evaluated epigenetic reprogramming in live, healthy clones indicate that although there is some variability between clones and their sexually-derived counterparts, these clones have undergone sufficient epigenetic reprogramming to carry out the coordinated functions necessary for survival and normal functioning. Molecular analyses reveal relatively small methylation differences, and either the animals are tolerant of such differences, or the epigenetic differences are below the threshold that poses observable adverse health outcomes.

C. Conclusions Regarding Food Consumption Risks

In order to evaluate potential food consumption risks associated with healthy-appearing clones, we developed a two-pronged approach. The first part of the approach is based on the hypothesis that a healthy animal is likely to be safe to eat, and relied on the CBSA. The second component of our two-pronged approach, or the *Compositional Analysis Approach*, assumed that if there are no material differences between the composition of milk and meat from animal clones (and their progeny) and their non-clone counterparts, then edible products derived from clone meat or milk would be as safe to eat as corresponding products from non-clones. This assessment assumed that animal clones and their progeny would be subject to all of the same federal and state requirements for milk and meat from conventionally bred animals.

Because each clone arises from an independent event, identification and characterization of potential subtle hazards (*e.g.,* alterations in gene expression, immune function, or hormone levels) is best accomplished by the evaluation of individual animals, at as fine a level of resolution as possible. Characterization of the overall functionality of clones, however, is likely best considered by evaluating the animal as a whole, in particular assessing the degree to which highly complex functions have been integrated, for example by demonstrating normal growth and successful reproduction.

The food consumption portion of the risk assessment postulated that because the only hazards that may be present in clones would arise from epigenetic dysregulation, and because only healthy animals meeting the same standards that conventional food animals or their edible products meet would be permitted for use as food, the only hazards that

could be present in these animals would be subtle. Part of the purpose of the CBSA approach was to determine whether any such subtle hazards could be identified. Following a detailed analysis of a wide range of health data, we identified only a few examples of altered physiological parameters in clones, and these were limited to young clone calves and swine. None of these alterations were correlated with any discernable adverse effects on animal health. We therefore concluded that no subtle hazards were identified that could pose food consumption risks from cattle, swine, or goat clones. The lack of allergenic and mutagenic effects in studies designed to detect those outcomes also indicated there were no food consumption risks.

Analyses of the composition of meat from bovine and swine clones and milk from bovine clones consistently indicate that there are no biologically relevant differences between the composition of food from clones, or their close comparators. In addition, there is no material difference, based on these studies, between the composition of meat and milk from clones and historical reference ranges of the composition of food from conventionally-bred animals.

D. Conclusions Regarding Food Consumption Risks from Clone Progeny

Progeny of animal clones are not anticipated to pose special animal health or food consumption concerns, as they are the product of sexual reproduction. The production of gametes by clones is expected to reset even those residual epigenetic reprogramming errors that could persist in healthy, reproducing clones. Studies in cattle and swine indicate that the progeny of clones are healthy and indistinguishable from other sexually-derived swine comparators. Because the value of clones lies in their genes, they are most likely to be used as breeding stock, and their use as food would be incidental. Almost all of the production animals (*i.e.,* sources of meat and milk) from the overall SCNT process are therefore likely to be sexually-reproduced progeny of clones. An extensive dataset on the progeny of swine clones indicates that the composition of meat from those animals does not differ materially from that of comparator animals or historical reference ranges.

E. Weight of Evidence Evaluations

As is the case for all risk assessments, the amount of data and information available on endpoints varied in quantity, with respect to both number of studies and number of data points available to evaluate. In some cases, evaluation of one endpoint indicated different outcomes in different laboratories. This Risk Assessment took such differences into account by applying the criteria listed above to consider the overall weight of evidence—that is, the extent to which the data supported each other, and where they did not, to

provide a framework for determining the underlying basis for the differences. Because the weight of evidence considers all of the information in one framework, it does not require any particular number of studies on any particular endpoint in order to be valid; rather, by considering all of the information together, it is able to develop a coherent perspective that takes into account biological assumptions, mechanisms, and empirical evidence. It also allows for the identification of uncertainties, and provides a science-based path for further investigations to resolve those uncertainties.

The judgment that cattle, swine, and goat clones meeting the same federal and state requirements as conventionally bred food animals would not likely pose food consumption risks, of course, contains some residual uncertainty. The source(s) of the uncertainty may be sorted into three categories:

1. Uncertainties associated with *empirical observations*. Uncertainties are lowest for those individual clones whose health has been thoroughly evaluated and, by inference, other clones produced using the same methodology. The uncertainties associated with the evaluation of empirical observations can be a function of the size, consistency, and quality of the data being evaluated. For example, the degree of confidence that can be placed in judgments arising from a well-conducted, consistent, and extensive dataset is much higher than from a small, poorly designed, and highly variable dataset. Further, because datasets tend to arise from an individual laboratory or producer, the uncertainties associated with that producer and method are lower than for other laboratories or producers for which less information is available.

2. Uncertainty stemming from *biological sources* can be minimized by the evaluation of the clones themselves. The most important factor in this evaluation is the healthy survival and functionality of individual clones, indicating that either the animal has minimal epigenetic dysregulation, or that any initial epigenetic dysregulation has been resolved. Uncertainty would be the lowest for individual clones demonstrating successful reproduction.

3. Uncertainties stemming from *technological or methodological* grounds encompass the degree to which judgments regarding clones arising from technologies in use when this risk assessment was conducted can be applied to modifications of the technology. These may only be resolved by the evaluation of the outcomes of those technological changes (*i.e.,* the actual clones).

Thus, our overall conclusions are:

For Animal Health: SCNT results in an increased frequency of health risks to animals involved in the cloning process, but these do not differ qualitatively from those observed in other ARTs or natural breeding. Cattle and sheep exhibit a set of clinical signs collectively referred to as LOS that do not appear to be present in swine or goats. Surrogate dams are at risk of complications from birth if the fetus suffers from LOS, or from accumulation of fluid in the placenta (hydrops). Clones exhibiting LOS may require additional supportive care at birth, but can recover and mature into normal, healthy animals. Most clones that survive the perinatal period are normal and healthy as determined by physiological measurements, behavior, and veterinary examinations. Progeny of animal clones also have been reported as normal and healthy.

For Food Consumption Risks: Extensive evaluation of the available data has not identified any food consumption risks or subtle hazards in healthy clones of cattle, swine, or goats. Thus, edible products from healthy clones that meet existing requirements for meat and milk in commerce pose no increased food consumption risk(s) relative to comparable products from sexually-derived animals. The uncertainties associated with this judgment are a function of the empirical observations and underlying biological processes contributing to the production of clones. Uncertainty about the health of clones decreases as they age and have more time to exhibit the full range of functionality expected of breeding stock. Edible products derived from the progeny of clones pose no additional food consumption risk(s) relative to corresponding products from other animals based on consistent empirical observations, underlying biological assumptions, and evidence from model systems.

Glossary

The following terms are defined as they are used within the current risk assessment. Unless otherwise indicated, definitions provided are the commonly accepted use of the term(s) at the Center for Veterinary Medicine, and may have been derived from various sources.[115]

allele	Any alternative form of a gene that can occupy a particular chromosomal locus.
anal atresia	Abnormally closed anal opening.
analyte	A substance undergoing analysis.
aneuploid	Describes a cell or organism which has an abnormal total number of chromosomes and where numbers of individual chromosomes are out of proportion with the numbers of the other chromosomes. Too many chromosomes is called hyperploidy; too few is called hypoploidy.
animal clones	Animals derived via somatic cell nuclear transfer techniques. The terminology employed in this assessment did not use "cloned animals." The phrase "cloned animals" does not clearly differentiate between the animal serving as the source of genome being propagated, or the animal that has been generated from a particular source. For example, the sentence "That field contains several cloned animals" does not specify whether the animals had been used as a source of material for SCNT or whether they had been generated by that technology.
ARTs	Assisted reproductive technologies.
biallelic	Referring to expression of two alleles at the same time.

[115] The various sources used for these definitions include: Dorland's *Medical Dictionary*, 30th Ed., W.B. Saunders Company, Philadelphia, 2003; Dictionary of Epidemiology. 3rd Ed. John M. Last. Oxford University Press, 1995; HTTP://bioethics.gov; http://biotech.icmb.utexas.edu; *Large Animal Internal Medicine*, 2nd Ed., Smith, B.P., Ed., Mosby – Year Book, Inc., St. Louis, 1996.; *Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses.* 7th Ed. Blood, D. C. and O. M. Radostits, Philadelphia: Bailliere Tindall Company, 1989. *The Merck Veterinary Manual,* 8th Ed. Online Version. C.M. Kanh and S Line, Ed. Merck & Co., Inc, NJ, 2003; and *The American Heritage® Dictionary of the English Language*, 4th Ed., Houghton Mifflin Company. 2002.

bioengineered animals The broadest category of animals associated with molecular biology techniques, including animal clones and all genetically engineered animals.

blastocyst An early stage in the development of mammalian embryos, when the embryo is a spherical body comprising an inner cell mass that will become the fetus and an outer ring of cells, the trophectoderm, that will become part of the placenta.

blastomere Any one of the cells formed from the first few cell divisions in animal embryology. The embryo usually divides into two, then four, then eight blastomeres, and so on.

Blastomere Nuclear Transfer (BNT) An assisted reproductive technique in which a blastomere is used as a donor for nuclear transfer into enucleated oöplasts.

capacitation The process of sperm maturation (or activation) that occurs post-ejaculation. Allows the spermatozoa to go through the acrosomal reaction in which factors in the sperm head that allow it to penetrate the egg are released and fertilize an oöcyte.

caruncle The site of attachment in the maternal uterus of the ruminant for the placental cotyledon (*see cotyledon*).

centromere (centromeric) A specialized chromosome region to which spindle fibers attach during cell division (mitosis) that is genetically inactive. This is constricted region of a mitotic chromosome that holds sister chromatids together—the crossing point in the "X" often used to depict chromosomes.

chimera An organism or recombinant DNA molecule created by joining DNA fragments from two or more different organisms.

chondrocyte A mature cartilage cell.

chorion The outermost membrane enclosing the fetus. It is formed from tissues on the outside of the embryo such as the trophoblast, and the part of it attached to the uterus wall eventually develops into the placenta.

chromatid One of the two daughter strands of a duplicated chromosome.

chromatin The network of fibers of DNA and protein that make up the chromosomes of the eukaryotic nucleus during interphase.

chromosome(s) A structure composed of one very long molecule of DNA and associated proteins (*e.g.* histones) that carries hereditary information.

cleavage The series of mitotic divisions by which a fertilized animal ovum changes, without any overall change in size, into a ball of smaller cells constituting the primitive embryo.

clone A group of cells or individuals that are genetically identical as a result of asexual reproduction including nuclear transfer.

cloning Asexual reproduction of animals using somatic cell nuclear transfer (SCNT).

coherence The extent to which a hypothesized causal association is compatible with preexisting theory and knowledge.

colostrum The first fluid secreted by the mammary glands at the time of birthing that is rich in antibodies and nutrients, and precedes the production of true milk. Its ingestion confers passive maternal immunity on the offspring of some species.

Comprehensive Veterinary Exam (CVE) Systematic approach for examining domestic livestock animals and making informed judgments as to their health. The CVE contains both objective and subjective information and is performed by a veterinarian.

congenital Existing at, and usually before, birth; referring to conditions that are present at birth, regardless of their causation.

consistency Close conformity between findings in different studies conducted by different methods or different investigators.

cortisol The major natural glucocorticoid hormone synthesized in the zona fasciculata of the adrenal cortex; it affects the metabolism of glucose, protein, and fats. It also regulates the immune system and affects many other functions.

cotyledon A lobule structure in ruminant placentae that form contact points between the fetal-derived placental tissues with the maternal caruncles (attachment points) of the uterus to form the functional units called placentomes. It consists mainly of a rounded mass of villi.

cryptorchid A male animal with one or both testicles retained within the body cavity.

cull	To remove unwanted members or parts from a herd.
cytoplasm	The living contents of the cell, exclusive of the nucleus, consisting of an aqueous protein matrix or gel, and where essential membranes and cellular organelles (mitochondria, plastids, etc.) reside.
de novo	Literally means "anew." Beginning a process from its origin with out prior plans.
dermatitis vegetans	A hereditary disease of the skin in swine (*see hyperkeratosis*).
differentiation	The process whereby relatively unspecialized cells, *e.g.* embryonic or regenerative cells, acquire specialized structural and/or functional features that characterize the cells, tissues, or organs of the mature organism or some other relatively stable phase of the organism's life history.
diploid	Having two sets of chromosomes.
DNA	Abbreviation for deoxyribonucleic acid; one of the two types of nucleic acids that constitutes the genetic material of most known organisms; usually in double helix form.
DNA polymerase	The enzyme responsible for copying DNA. Common name for either of two categories of enzymes that catalyze the synthesis of DNA from deoxyribonucleoside triphosphates in the presence of a nucleic-acid primer.
ductus arteriosus	The blood vessel between the pulmonary artery (carries blood from the heart to the lungs for oxygenation) and the aorta (carries oxygenated blood to the rest of the body). During gestation the ductus arteriosus bypasses the fetal lungs, and is normally sealed after birth.
ductus venosus	The blood vessel between the umbilical vein and the caudal vena cava (carries oxygenated blood from the dam, bypassing the liver, through the vena cava to the heart of the fetus). It is normally sealed shortly after birth.
dysregulate	Abnormal or impaired control of gene expression.
dystocia	Abnormal or difficult labor.

ectoderm The outermost layer of tissue in a developing embryo that will eventually become the skin and/or other outer surface of the organism, the outermost parts of the nervous system, and various other outer and external organs depending on the organism.

embryo In mammals, the term is restricted to the structure present in the early part of gestation that develops into a fetus.

embryo cloning Another term for blastomere nuclear transfer.

empirical That which can be seen or observed alone, often without reliance on theory.

endoderm The innermost layer of tissue in a developing animal embryo that will eventually become the digestive tract, respiratory tract, and various other things depending on the organism.

enucleate Removal of an organ or mass from its supporting tissues.

epigenetic Describing any of the mechanisms regulating the expression and interaction of genes, particularly during the development process. These include changes that influence the phenotype but have arisen as a result of mechanisms such as inherited patterns of DNA methylation rather than differences in gene sequence: imprinting is an example of this.

epigenetic reprogramming In the case of somatic cell nuclear transfer (SCNT), the process of altering the instructions governing the expression of genes in the chromosomal DNA of the donor cell such that embryonic or totipotent (able to differentiate along any line or into any type of cell) gene expression conditions are reestablished.

epigenetic variation/effects Non-hereditary, phenotypic changes in the expression in a single gene.

estrous Pertaining to estrus. (Adjective)

estrus The recurrent, restricted period of sexual receptivity in female mammals other than human females, marked by intense sexual urge. (Noun)

euchromatin One of two types of chromatin seen during interphase of the cell cycle. It is genetically active (transcription occurs in it) and less condensed than heterochromatin (the other type of chromatin).

eukaryote An organism whose cells have a true nucleus, *i.e.*, one bounded by a

	nuclear membrane, within which lie the chromosomes, combined with proteins and exhibiting mitosis; eukaryotic cells also contain many membrane-bound compartments (organelles) in which cellular functions are performed.
F_1	Abbreviation for filial generation 1 (first generation). The initial hybrid generation resulting from a cross between two parents.
farrow	In swine, the process of giving birth. Also used to describe a litter of pigs.
fat cow syndrome	A multifactorial disease condition often occurring in dairy cows following parturition; associated with excessive mobilization of fat to the liver in well-conditioned cows. This mobilization is induced by the negative energy balance and hormonal changes. Presenting signs usually include depression, anorexia, weight loss, and weakness that can lead to recumbency.
fecundity	The physiological ability to reproduce, as opposed to fertility.
fertility	The capacity to conceive or induce conception.
foramen ovale	A hole in the fetal heart between the right and left atria, for the purpose of bypassing the lungs. It is normally sealed shortly after birth.
founder animal	An organism that serves as the progenitor of a particular lineage.
freemartin	A sexually maldeveloped female calf born as a twin to a normal male calf. The reproductive tract hypoplasia results in an infantile uterus that does not develop appropriately with the growth of the rest of the calf and fails to respond to puberty. It is commonly sterile and intersexual as the result of male hormones reaching it through shared placental blood vessels.
gamete	A mature reproductive cell capable of fusing with a cell of similar origin but of opposite sex to form a zygote from which a new organism can develop. Gametes normally have haploid chromosome content. In animals, a gamete is a sperm or egg.
gametogenesis	The process of the formation of gametes.
gene expression	The process by which a cell transcribes the information stored in its genome to carry out the functions of life.
genetic	The process of rearranging the genome of the nucleus to restore a

reprogramming	cell's totipotency so it can differentiate into different types of cells and develop into a whole organism. Also known as de-differentiation.
genetically engineered animals	A subset of animals associated with molecular biology techniques. Includes transgenic animals, animals subjected to gene therapy and mosaic animals. This subset does not include animal clones.
genome	The full set of genes in an individual, either haploid (the set derived from one parent) or diploid (the set derived from both parents).
genotype	The entire genetic constitution of an individual.
germ cell	A reproductive cell such as a spermatocyte or an oöcyte, or a cell that will develop into a reproductive cell.
gilt	A female pig that is intended for breeding but has not yet given birth.
gonadotropin	Any hormone that stimulates the testes or ovaries.
haploid	An individual or cell having only one member of each pair of homologous chromosomes.
harm	An adverse outcome.
hazard	Something that can produce harm.
heifer	A female bovine that has not yet produced a calf.
hematology	The branch of medicine that deals with the blood and blood-forming tissues.
hemogram	A written record or graphic representation of a detailed blood assessment such as the complete blood count or differential leukocyte count.
hermaphrodite	An individual characterized by the presence of both male and female sex organs. The condition is caused by an anomalous differentiation of the gonads: an animal with ambiguous genitalia, typically a penis with ovaries or a vulva with testicles
heterochromatin	The condensed and genetically inactivated portion of a chromosome.
histones	Chromatin proteins commonly associated with the DNA of somatic

	cells in eukaryotes and they are involved in packaging of the DNA and the regulation of gene activity.
hormone	A chemical substance produced in the body by an organ, cells of an organ, or scattered cells, having a specific regulatory effect on the activity of an organ or organs. The term was originally applied to substances secreted by endocrine glands and transported in the bloodstream to distant target organs, but later it was applied to various substances having similar actions but not produced by special glands.
hydroallantois	Abnormal fluid accumulation in the allantoic cavity of the placenta. (*See hydrops*.)
hydrops	Edema. Hydrops refers to a set of conditions relating to abnormal fluid accumulation in one or more compartments of the placenta and/or the fetus itself, and are alternatively referred to as hydroallantois, hydramnios or hydrops fetalis, depending on where the edema occurs.
hyperkeratosis	Characterized by lesions of the superficial layers of the epidermis. These lesions rapidly become covered with scales, and then develop hard, dry crusts with deep fissures. Generally referred to as parakeratosis in swine.
hypoplasia	Incomplete development or underdevelopment of an organ or tissue.
hypospadius	A developmental anomaly in which the urethra opens inferior (below) to its usual location; usually seen in males with the opening on the underside of the penis or on the perineum.
imprinted genes	Those genes whose degree of expression is determined by their derivation from either the dam or the sire.
in vitro	Outside the organism, or in an artificial environment. This term applies, for example, to cells, tissues or organs cultured in glass or plastic containers.
in vivo	Literally means "in life;" a biologic or biochemical process occurring within a living organism.
inner cell mass	The group of cells in a blastocyst that are destined to form the fetus.
inner cell mass (ICM)	A cluster of cells within the blastocyst. The inner cell mass will form all of the tissues of the organism and these cells are pluripotent.

ketonuria Ketone bodies in the urine, as in diabetes mellitus; called also acetonuria and hyperketonuria.

ketosis A metabolic disease of lactating dairy cows characterized by weight loss, decreased milk production, and neurologic abnormalities that usually occur during the first 6 weeks of lactation.

Large Offspring Syndrome (LOS) A morphologic syndrome presumably expressed at the molecular and physiological level due to some alterations in embryonic gene expression. Animal clones with LOS may experience difficulties in developing and maintaining the placenta. An LOS fetus is unusually large for its species, has longer than usual gestation periods, and often has immature lungs or heart abnormalities. Kidneys and liver may also be affected.

leukocytosis A transient increase in the number of leukocytes (white blood cells) in the blood.

leukopenia A reduction in the number of leukocytes in the blood.

locus The specific site of a gene on a chromosome.

long terminal repeats A double-stranded sequence, generally several hundred base pairs long, at the two ends of the genetic sequence of retroviruses.

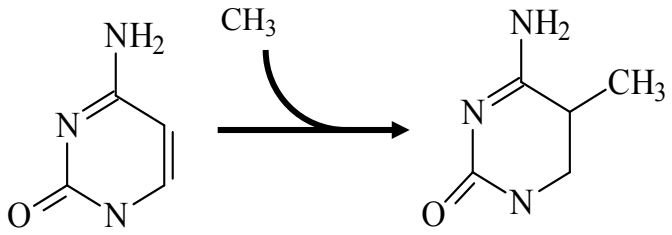
mastitis Inflammation of the mammary gland or breast.

meconium First stool in the intestine of a full-term fetus.

meiosis The process in which a single diploid cell becomes four haploid cells in two consecutive divisions of the nucleus of an eukaryotic cell. In multicellular higher organisms this occurs only in the progenitors of sex cells and never in somatic cells.

methylation The addition of a methyl group ($-CH_3$) to a larger molecule (e.g. cytosine methylation)

cytosine 5-methyl cytosine



metritis Inflammation of the uterus.

mitosis The division of a eukaryotic cell nucleus to produce two daughter nuclei that contain identical numbers of chromosomes and that are identical genetically to the parent nucleus except where crossing over or mutation has occurred.

monozygotic twin One of a pair of twins derived from a single fertilized egg or zygote. *Synonym*: identical twin.

morphology The form and structure of an organism, organ, or part.

morula The solid mass of blastomeres formed from the cleavage of a fertilized ovum or egg.

murine Pertaining to or affecting mice or rats.

neoplasia Abnormal and uncontrolled cell growth that often produces a tumor (a *neoplasm*) that may or may not be cancerous (*i.e.*, capable of spread or metastasis).

nuclear transfer Transferring the nucleus with its chromosomal DNA from one (donor) cell to another (recipient) cell.

nucleic acids A large molecule composed of nucleotide subunits. DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) are examples.

nucleoside A molecule composed of a purine or pyrimidine nitrogenous base attached to the five-carbon sugar. This glycosylamine is a component of nucleic acids.

nucleotide A molecule composed of a purine or pyrimidine nitrogenous base attached to the five-carbon sugar which also has a phosphate group attached to it. It is the constitutional unit into which nucleic acids are broken down by partial hydrolysis and from which they are built.

nucleus	The most conspicuous organelle of a eukaryotic cell; it contains the chromosomes and is the site of genomic DNA replication and or RNA synthesis in the cell.
oöcyte	A cell of an animal ovary that undergoes meiosis to form an ovum.
oöplasmic remodeling	After nuclear transfer, the cytoplasm of the oöcyte (oöplasm) alters the morphology of the nucleus, so that it more closely resembles the nucleus of an embryo.
oöplast	The remaining portion of the oöcyte following enucleation.
oviduct	A tube from the ovary to the uterus through which ova (eggs) may pass.
ovum	The female reproductive cell which, after fertilization, becomes a zygote that develops into a new member of the same species. Also called an egg.
parakeratosis	A nutritional deficiency disease of 6- to 16-wk-old pigs that is characterized by lesions of the superficial layers of the epidermis. It is a metabolic disturbance resulting from a deficiency of zinc or an excess of calcium in the diet.
parity	The condition of having given birth.
parthenogenesis	The development of a new individual from an unfertilized female gamete.
parturition	The act or process of giving birth to offspring.
patent ductus arteriosus	The failure of the ductus arteriosus to close after birth resulting in extra blood flow to the lungs and recirculation of oxygenated blood to the lungs rather than the rest of the body.
patent urachus	The failure or the urachus to close during parturition, resulting in the inability to excrete urinary waste.
phagocytosis	The uptake of extracullular materials by the formation of a pocket from the cellular membrane and its subsequent pinching off.
phenotype	The totality of the observable functional and structural characteristics of an organism as determined by its genotype and its interaction with its environment.

phytate(s) A form of phosphorus commonly occurring in grain products, which is indigestible in non-ruminant species.

placentomes Placental junctures consisting of the uterine caruncle and the placental cotyledon, which permits vascular transport of nutrients into and waste out of the fetal environment.

ploidy Degree of repetition of the basic number of chromosomes.

pluripotent Capable of differentiating into more than one cell type.

polar body A small cell containing little cytoplasm that is the by-product of oöcyte meiosis in female animals.

polycythemia An increase in the total red cell mass of the blood.

polymorphism Describes a substance that can take on several different forms. Can refer to subtle differences in DNA sequences among individuals. It also may refer to a protein which can be coded by several different sequences; these variations do not ruin the protein's function.

polyploidy The state of a cell having more than two times the haploid number of chromosomes in its nucleus.

portal Anatomical nomenclature pertaining to an opening, especially the site of entrance to an organ of the blood vessels and other structures supplying or draining it.

predation The capturing and consumption of prey as a means of maintaining life.

pregnancy toxemia A pathologic metabolic disturbance of pregnancy that results when fetal carbohydrate or energy demand exceeds the maternal supply during the last trimester of pregnancy. Specific to sheep and goats.

preimplantation A period very early in embryo development, before the embryo attaches to the uterus.

progeny An animal derived from sexual reproduction that has at least one cloned animal as a parent (but could result from two cloned animals mating).

promoter A sequence of the DNA molecule to which RNA polymerase will bind and initiate transcription.

promoter A segment of DNA acting as a controlling element in the expression of a gene.

promoter-enhancer sequence A control element that can increase expression of a gene.

pronucleus The pronucleus is the structure that contains the haploid genome of the sperm or ovum after fertilization occurs, but before they fuse to make the nucleus of the zygote, or the single-celled diploid organism.

p-value A measure of the probability that a difference between groups during an experiment happened by chance.

recumbancy Lying down.

rendering Reducing, converting, or melting down animal by-products by heating; a cooking and drying process that yields fat of varying grades, both edible and inedible (depending on raw material source), and animal protein that is useful for animal feeds and fertilizer.

risk A set of conditions that links an exposure to the likelihood of an adverse outcome.

risk assessment The methodology used to characterize potential risks and the conditions that result in the potential to experience risk.

risk management The set of activities applied to identify and evaluate alternative strategies (often regulatory), and select among them on the basis of economic, political, scientific, ethical and social conditions or criteria.

RNA Abbreviation for ribonucleic acid that serves to carry information from DNA to other parts of the cell or that has other functions. The generation of messenger RNA is a critical step in gene expression.

RNA polymerase An enzyme that transcribes the information in a DNA sequence into RNA.

ruminant Animals having a rumen - a large digestive sac in which fibrous plant material is fermented by commensal microbes, prior to its digestion in a "true" stomach (the *abomasum*). Common farm ruminants are cattle and sheep.

SCNT Acronym for Somatic Cell Nuclear Transfer. The process of generating a live organism asexually by transferring the diploid

	nucleus of a somatic cell from a donor animal to the enucleated embryo of a recipient animal.
scours	Severe diarrhea in farm animals.
senescence	The process or condition of growing old in which cells, tissues, and organisms deteriorate and finally die.
sequellae	Morbid conditions occurring as a consequence of another condition or event.
sexual reproduction	The production of offspring by the fusion of male and female gametes (in contrast to 'asexual reproduction').
somatic cell	Any cell of an organism other than a germ cell.
stem cell	A totipotent or pluripotent cell that can replicate indefinitely and which can differentiate into other cells; stem cells serve as a continuous source of new cells.
stochastic	Pertaining to a random process, used particularly to refer to a time series of random variables. Arrived at by skillful conjecture; *e.g.* a stochastic model, a stochastic process.
superovulate	To produce numerous ova at one time.
telomerase	A DNA polymerase enzyme that maintains the structure of the telomere by adding the required repetitive sequences to the ends of eukaryotic chromosomes.
telomere	The structure that seals the end of a chromosome.
tetraploid	An organism or cell containing four haploid sets of chromosomes (see polyploidy).
totipotent	Capable of becoming any cell type in the body.
transcription	The process by which a single-stranded RNA with a base sequence complementary to one strand of a double-stranded DNA is synthesized.
transgenic	Contains heritable DNA from another source. A transgenic animal is one that has been intentionally altered using molecular biology techniques that result in heritable changes (insertions, deletions or rearrangements) in the nucleic acid sequence of the nucleus or mitochondria, and includes any offspring that inherit those changes.

translation	The second major step of gene expression in which the particular sequence of bases in the transcribed mRNA determines the sequence of amino acids in the proteins (or polypeptides) being synthesized (see transcription).
transposable element	A genetic element that has the ability to move (transpose) from one site on a chromosome to another.
trophectoderm	The group of cells in the blastocyst that form the placenta and other non-fetal tissues.
trophoblast	A layer of extra-embryonic ectodermal tissue on the outside of the blastocyst. It attaches the blastocyst to the endometrium of the uterine wall and supplies nutrition to the embryo.
urachus	A structure through which a fetus excretes urinary waste. In normal development, this structure would close at the time of parturition.
ventricle (ventriculus)	A small cavity or chamber within a body or organ, especially: (a) the chamber on the left side of the heart that receives oxygenated arterial blood from the left atrium and contracts to force it into the aorta; and (b) the chamber on the right side of the heart that receives deoxygenated venous blood from the right atrium and forces it into the pulmonary artery.
villi	Microscopic vascular protrusions from the surface of a membrane.
wild type	The phenotype that is characteristic of most of the members of a species occurring naturally and contrasting with the phenotype of a mutant.
xist	Enzyme that deactivates one of the two X chromosomes in female embryos.
zona pellucida	The thick, transparent, non-cellular outer layer surrounding an oöcyte and fertilized ovum.
zygote	The diploid cell that results from the union of a sperm cell and an egg cell.

References

(2006) *Essential Rendering: All About The Animal By-Products Industry*. National Renderers Association: Arlington, VA

(2006) *Veterinary Values*. Veterinary Medicine Publishing group:

Adamec V, Cassell BG, Smith EP, Pearson RE (2006) Effects of inbreeding in the dam on dystocia and stillbirths in US Holsteins. *J Dairy Sci* **89**: 307-314

Agca Y, Monson RL, Northey DL, Mazni OA, Schaefer DM, Rutledge JJ (1998) Transfer of fresh and cryopreserved IVP bovine embryos: normal calving, birth weight and gestation lengths. *Theriogenology* **50**: 147-162

Aherne FX, Kirkwood RN (1985) Nutrition and sow prolificacy. *J Reprod Fertil Suppl* **33**: 169-183

Ahlschwede W.T. and Kuhlers D. Guidelines for choosing replacement females. Purdue University Cooperative Extension Service . 1992.
Ref Type: Electronic Citation

Akagi S, Adachi N, Matsukawa K, Kubo M, Takahashi S (2003) Developmental potential of bovine nuclear transfer embryos and postnatal survival rate of cloned calves produced by two different timings of fusion and activation. *Mol Reprod Dev* **66**: 264-272

Allegrucci C, Thurston A, Lucas E, Young L (2005) Epigenetics and the germline. *Reproduction* **129**: 137-149

Allen JF, Allen CA (1999) A mitochondrial model for premature ageing of somatically cloned mammals. *IUBMB Life* **48**: 369-372

Allen WR (2005) The development and application of the modern reproductive technologies to horse breeding. *Reprod Domest Anim* **40**: 310-329

Ambrose DJ, Kastelic JP, Corbett R, Pitney PA, Petit HV, Small JA, Zalkovic P (2006) Lower pregnancy losses in lactating dairy cows fed a diet enriched in alpha-linolenic acid. *J Dairy Sci* **89**: 3066-3074

Anderson S, de Bruijn MH, Coulson AR, Eperon IC, Sanger F, Young IG (1982) Complete sequence of bovine mitochondrial DNA. Conserved features of the mammalian mitochondrial genome. *J Mol Biol* **156**: 683-717

Aoki F, Worrad DM, Schultz RM (1997) Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. *Dev Biol* **181**: 296-307

Aoki S, Takahashi R, Nisisouzu T, Kitamura S, Dochi O, Kishi M, Morita S, Komiya M, Tarawaki Y, Hoyama H (2003) A comparative investigation of the characteristics of Holstein coes cloned from colostrum-derived mammary grand epithelial cells in an automatic milking system [ABSTRACT ONLY]. *Theriogenology* **59**: 234

Apimeteetumrong M, Thuangsanthia A, Leingcharoen N, Yiengvisavakul V, Harintharanon A, Kunavongkrit A, Sumretprasong J, Vignon X, Techakumphu M (2004) The effect of activation protocols on the development of cloned goat embryos. *J Vet Med Sci* **66**: 1529-1534

Archer GS, Dindot S, Friend TH, Walker S, Zaunbrecher G, Lawhorn B, Piedrahita JA (2003) Hierarchical phenotypic and epigenetic variation in cloned swine. *Biol Reprod* **69**: 430-436

Archer GS, Friend TH, Piedrahita J, Nevill CH, Walker S (2003) Behavioral variation among cloned pigs. *Applied Animal Behaviour Science* **82**: 151-161

Arlington, K. Clone death a mystery. News.com.au . 11-18-2003.
Ref Type: Internet Communication

Armstrong L, Lako M, Dean W, Stojkovic M (2006) Epigenetic modification is central to genome reprogramming in somatic cell nuclear transfer. *Stem Cells* **24**: 805-814

Arnold DR, Bordignon V, Lefebvre R, Murphy BD, Smith LC (2006) Somatic cell nuclear transfer alters peri-implantation trophoblast differentiation in bovine embryos. *Reproduction* **132**: 279-290

Aston KI, Li GP, Hicks BA, Sessions BR, Pate BJ, Hammon D, Bunch TD, White KL (2006) Effect of the time interval between fusion and activation on nuclear state and development in vitro and in vivo of bovine somatic cell nuclear transfer embryos. *Reproduction* **131**: 45-51

Aston KI, Li GP, Hicks BA, Sessions BR, Pate BJ, Hammon DS, Bunch TD, White KL (2006) The developmental competence of bovine nuclear transfer embryos derived from cow versus heifer cytoplasts. *Anim Reprod Sci* **95**: 234-243

Auldist MJ, Johnston KA, White NJ, Fitzsimons WP, Boland MJ (2004) A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. *J Dairy Res* **71**: 51-57

Avner P, Heard E (2001) X-chromosome inactivation: counting, choice and initiation. *Nat Rev Genet* **2**: 59-67

Bacon SJ, Ellis SA, Antczak DF (2002) Control of expression of major histocompatibility complex genes in horse trophoblast. *Biol Reprod* **66**: 1612-1620

Baguisi A, Behboodi E, Melican DT, Pollock JS, Destrempes MM, Cammuso C, Williams JL, Nims SD, Porter CA, Midura P, Palacios MJ, Ayres SL, Denniston RS, Hayes ML, Ziomek CA, Meade HM, Godke RA, Gavin WG, Overstrom EW, Echelard Y (1999) Production of goats by somatic cell nuclear transfer. *Nat Biotechnol* **17**: 456-461

Baillargeon P, Fecteau G, Pare J, Lamothe P, Sauve R (2001) Evaluation of the embryo transfer procedure proposed by the International Embryo Transfer Society as a method of controlling vertical transmission of Neospora caninum in cattle. *J Am Vet Med Assoc* **218**: 1803-1806

Barlow SM, Greig JB, Bridges JW, Carere A, Carpy AJ, Galli CL, Kleiner J, Knudsen I, Koeter HB, Levy LS, Madsen C, Mayer S, Narbonne JF, Pfannkuch F, Prodanchuk MG, Smith MR, Steinberg P (2002) Hazard identification by methods of animal-based toxicology. *Food Chem Toxicol* **40**: 145-191

Basrur PK, King WA (2005) Genetics then and now: breeding the best and biotechnology. *Rev Sci Tech* **24**: 31-49

Batchelder CA, Bertolini M, Mason JB, Moyer AL, Hoffert KA, Petkov SG, Famula TR, Angelos J, George LW, Anderson GB (2007) Perinatal physiology in cloned and normal calves: hematologic and biochemical profiles. *Cloning Stem Cells* **9**: 83-96

Batchelder CA, Bertolini M, Mason JB, Moyer AL, Hoffert KA, Petkov SG, Famula TR, Angelos J, George LW, Anderson GB (2007) Perinatal physiology in cloned and normal calves: physical and clinical characteristics. *Cloning Stem Cells* **9**: 63-82

Batchelder CA, Hoffert KA, Bertolini M, Moyer AL, Mason JB, Petkov SG, Famula TR, Anderson GB (2005) Effect of the nuclear-donor cell lineage, type, and cell donor on development of somatic cell nuclear transfer embryos in cattle. *Cloning Stem Cells* **7**: 238-254

Bavister BD (2006) The mitochondrial contribution to stem cell biology. *Reprod Fertil Dev* **18**: 829-838

Bazer FW, Spencer TE (2005) Reproductive biology in the era of genomics biology. *Theriogenology* **64** : 442-456

Bearden H, Fuquay J (1980) *Applied animal reproduction*. Prentice-Hall:

Bearden HJ, Fuquay JW (2000) *Applied Animal Reproduction*. Prentice-Hall, Inc.: Upper Saddle River

Beaujean N, Martin C, Debey P, Renard JP (2005) [Reprogramming and epigenesis]. *Med Sci (Paris)* **21** : 412-421

Beaujean N, Taylor J, Gardner J, Wilmut I, Meehan R, Young L (2004) Effect of limited DNA methylation reprogramming in the normal sheep embryo on somatic cell nuclear transfer. *Biol Reprod* **71**: 185-193

Behboodi E, Anderson GB, BonDurant RH, Cargill SL, Kreuscher BR, Medrano JF, Murray JD (1995) Birth of large calves that developed from in-vitro derived bovine embryos. *Theriogenology* **44**: 227-232

Behboodi E, Ayres SL, Memili E, O'Coin M, Chen LH, Reggio BC, Landry AM, Gavin WG, Meade HM, Godke RA, Echelard Y (2005) Health and reproductive profiles of malaria antigen-producing transgenic goats derived by somatic cell nuclear transfer. *Cloning and Stem Cells* **7**: 107-118

Bellows RA, Lammoglia MA (2000) Effects of severity of dystocia on cold tolerance and serum concentrations of glucose and cortisol in neonatal beef calves. *Theriogenology* **53**: 803-813

Berg DK, Li C, Asher G, Wells DN, Oback B (2007) Red Deer Cloned from Antler Stem Cells and Their Differentiated Progeny. *Biol Reprod*

Bernstein JA, Bernstein IL, Bucchini L, Goldman LR, Hamilton RG, Lehrer S, Rubin C, Sampson HA (2003) Clinical and laboratory investigation of allergy to genetically modified foods. *Environ Health Perspect* **111**: 1114-1121

Bertolini M, Anderson GB (2002) The placenta as a contributor to production of large calves. *Theriogenology* **57**: 181-187

Bertolini M, Mason JB, Beam SW, Carneiro GF, Sween ML, Kominek DJ, Moyer AL, Famula TR, Sainz RD, Anderson GB (2002) Morphology and morphometry of in vivo- and in vitro-produced bovine concepti from early pregnancy to term and association with high birth weights. *Theriogenology* **58**: 973-994

Bertolini M, Moyer AL, Mason JB, Batchelder CA, Hoffert KA, Bertolini LR, Carneiro GF, Cargill SL, Famula TR, Calvert CC, Sainz RD, Anderson GB (2004) Evidence of increased substrate availability to in vitro-derived bovine foetuses and association with accelerated conceptus growth. *Reproduction* **128**: 341-354

Bertolini M, Wallace CR, Anderson GB (2006) Expression profile and protein levels of placental products as indirect measures of placental function in in vitro-derived bovine pregnancies. *Reproduction* **131**: 163-173

Besser TE, Szenci O, Gay CC (1990) Decreased colostral immunoglobulin absorption in calves with postnatal respiratory acidosis. *J Am Vet Med Assoc* **196**: 1239-1243

Betthauser J, Forsberg E, Augenstein M, Childs L, Eilertsen K, Enos J, Forsythe T, Golueke P, Jurgella G, Koppang R, Lesmeister T, Mallon K, Mell G, Misica P, Pace M, Pfister-Genskow M, Strelchenko N, Voelker G, Watt S, Thompson S, Bishop M (2000) Production of cloned pigs from in vitro systems. *Nat Biotechnol* **18**: 1055-1059

Betthauser JM, Pfister-Genskow M, Xu H, Golueke PJ, Lacson JC, Koppang RW, Myers C, Liu B, Hoeschele I, Eilertsen KJ, Leno GH (2006) Nucleoplasmin facilitates reprogramming and in vivo development of bovine nuclear transfer embryos. *Mol Reprod Dev* **73**: 977-986

Betts D, Bordignon V, Hill J, Winger Q, Westhusin M, Smith L, King W (2001) Reprogramming of telomerase activity and rebuilding of telomere length in cloned cattle. *Proc Natl Acad Sci U S A* **98**: 1077-1082

Betts DH, King WA (2001) Genetic regulation of embryo death and senescence. *Theriogenology* **55**: 171-191

Betts DH, Perrault SD, Petrik J, Lin L, Favetta LA, Keefer CL, King WA (2005) Telomere length analysis in goat clones and their offspring. *Mol Reprod Dev* **72**: 461-470

Beyhan Z, Forsberg EJ, Eilertsen KJ, Kent-First M, First NL (2007) Gene expression in bovine nuclear transfer embryos in relation to donor cell efficiency in producing live offspring. *Mol Reprod Dev* **74**: 18-27

Beyhan Z, Ross PJ, Iager AE, Kocabas AM, Cunniff K, Rosa GJ, Cibelli JB (2007) Transcriptional reprogramming of somatic cell nuclei during preimplantation development of cloned bovine embryos. *Dev Biol*

Bhak JS, Lee SL, Ock SA, Mohana KB, Choe SY, Rho GJ (2006) Developmental rate and ploidy of embryos produced by nuclear transfer with different activation treatments in cattle. *Anim Reprod Sci* **92**: 37-49

Bhojwani S, Tomek W, Jonas L, Becker F, Alm H, Torner H, Kanitz W, Poehland R (2007) Ultrastructural analysis reveals striking differences of intercellular contact lengths in bovine embryos produced in vivo, in vitro and by somatic cell nuclear transfer. *Mol Reprod Dev* **74**: 775-784

Bhojwani S, Vajta G, Callesen H, Roschlau K, Kuwer A, Becker F, Alm H, Torner H, Kanitz W, Poehland R (2005) Developmental competence of HMC(TM) derived bovine cloned embryos obtained from somatic cell nuclear transfer of adult fibroblasts and granulosa cells. *J Reprod Dev* **51**: 465-475

Bird A (2002) DNA methylation patterns and epigenetic memory. *Genes Dev* **16**: 6-21

Bishop MD (2000) Cloned pig litter update. *Nat Biotechnol* **18**: 1227

Bjerregaard B, Pedersen HG, Jakobsen AS, Rickords LF, Lai L, Cheong HT, Samuel M, Prather RS, Strejcek F, Rasmussen ZR, Laurincik J, Niemann H, Maddox-Hyttel P, Thomsen PD (2007) Activation of ribosomal RNA genes in porcine embryos produced in vitro or by somatic cell nuclear transfer. *Mol Reprod Dev* **74**: 35-41

Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW (1997) Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* **91**: 25-34

Blaxter K (1989) *Energy Metabolism in Animals and Man*. Cambridge University Press: New York

Blelloch R, Wang Z, Meissner A, Pollard S, Smith A, Jaenisch R (2006) Reprogramming efficiency following somatic cell nuclear transfer is influenced by the differentiation and methylation state of the donor nucleus. *Stem Cells* **24**: 2007-2013

Blelloch RH, Hochedlinger K, Yamada Y, Brennan C, Kim M, Mintz B, Chin L, Jaenisch R (2004) Nuclear cloning of embryonal carcinoma cells. *Proc Natl Acad Sci U S A* **101**: 13985-13990

Block J, Drost M, Monson RL, Rutledge JJ, Rivera RM, Paula-Lopes FF, Ocon OM, Krininger CE, III, Liu J, Hansen PJ (2003) Use of insulin-like growth factor-I during embryo culture and treatment of recipients with gonadotropin-releasing hormone to increase pregnancy rates following the transfer of in vitro-produced embryos to heat-stressed, lactating cows. *J Anim Sci* **81**: 1590-1602

Blood DC, Radostits OM (1989) *Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*. Bailliere Tindall: Philadelphia

Boiani M, Eckardt S, Scholer HR, McLaughlin KJ (2002) Oct4 distribution and level in mouse clones: consequences for pluripotency. *Genes Dev* **16**: 1209-1219

Boiani M, Gentile L, Gambles VV, Cavaleri FM, Redi CA, Scholer HR (2005) Variable 'reprogramming' of the pluripotent stem cell marker Oct4 in mouse clones: distinct developmental potentials in different culture environments. *Stem Cells*

Bonczek RR, Richardson DO, Moore ED, Miller RH, Owen JR, Dowlen HH, Bell BR (1992) Correlated response in growth and body measurements accompanying selection for milk yield in Jerseys. *J Dairy Sci* **75**: 307-316

Bondioli K, Ramsoondar J, Williams B, Costa C, Fodor W (2001) Cloned pigs generated from cultured skin fibroblasts derived from a H-transferase transgenic boar. *Mol Reprod Dev* **60** : 189-195

Booth PJ, Tan SJ, Holm P, Callesen H (2001) Application of the zona-free manipulation technique to porcine somatic nuclear transfer. *Cloning Stem Cells* **3**: 191-197

Booth PJ, Tan SJ, Reipurth R, Holm P, Callesen H (2001) Simplification of bovine somatic cell nuclear transfer by application of a zona-free manipulation technique. *Cloning Stem Cells* **3**: 139-150

Boquest AC, Grupen CG, Harrison SJ, McIlfatrick SM, Ashman RJ, d'Apice AJF, Nottle MB (2002) Production of cloned pigs from cultured fetal fibroblast cells. *Biol Reprod* **66**: 1283-1287

Bordignon V, Smith LC (2006) Telophase-stage host ooplasts support complete reprogramming of roscovitine-treated somatic cell nuclei in cattle. *Cloning Stem Cells* **8**: 305-317

Borowczyk E, Caton JS, Redmer DA, Bilski JJ, Weigl RM, Vonnahme KA, Borowicz PP, Kirsch JD, Kraft KC, Reynolds LP, Grazul-Bilska AT (2006) Effects of plane of nutrition on in vitro fertilization and early embryonic development in sheep. *J Anim Sci* **84**: 1593-1599

Bortvin A, Eggan K, Skaletsky H, Akutsu H, Berry DL, Yanagimachi R, Page DC, Jaenisch R (2003) Incomplete reactivation of Oct4-related genes in mouse embryos cloned from somatic nuclei. *Development* **130**: 1673-1680

Bosch P, Pratt SL, Stice SL (2006) Isolation, characterization, gene modification, and nuclear reprogramming of porcine mesenchymal stem cells. *Biol Reprod* **74**: 46-57

Bourc'his D, Le Bourhis D, Patin D, Niveleau A, Comizzoli P, Renard JP, Viegas-Pequignot E (2001) Delayed and incomplete reprogramming of chromosome methylation patterns in bovine cloned embryos. *Curr Biol* **11**: 1542-1546

Bousquet D, Blondin P (2004) Potential uses of cloning in breeding schemes: dairy cattle. *Cloning Stem Cells* **6**: 190-197

Bowles EJ, Campbell KH, St John JC (2007) Nuclear transfer: preservation of a nuclear genome at the expense of its associated mtDNA genome(s). *Curr Top Dev Biol* **77**: 251-290

Brambrink T, Hochedlinger K, Bell G, Jaenisch R (2006) ES cells derived from cloned and fertilized blastocysts are transcriptionally and functionally indistinguishable. *Proc Natl Acad Sci U S A* **103**: 933-938

Breazile JE, Vollmer LA, Rice LE (1988) Neonatal adaptation to stress of parturition and dystocia. *Vet Clin North Am Food Anim Pract* **4**: 481-499

Breukelman SP, Reinders JM, Jonker FH, de Ruigh L, Kaal LM, van Wagtendonk-de Leeuw AM, Vos PL, Dieleman SJ, Beckers JF, Perenyi Z, Taverne MA (2004) Fetometry and fetal heart rates between Day 35 and 108 in bovine pregnancies resulting from transfer of either MOET, IVP-co-culture or IVP-SOF embryos. *Theriogenology* **61**: 867-882

Brown DT, Herbert M, Lamb VK, Chinnery PF, Taylor RW, Lightowlers RN, Craven L, Cree L, Gardner JL, Turnbull DM (2006) Transmission of mitochondrial DNA disorders: possibilities for the future. *Lancet* **368**: 87-89

Bruggerhoff K, Zakhartchenko V, Wenigerkind H, Reichenbach HD, Prelle K, Schernthaner W, Alberio R, Kuchenhoff H, Stojkovic M, Brem G, Hiendleder S, Wolf E (2002) Bovine somatic cell nuclear transfer using recipient oocytes recovered by ovum pick-up: effect of maternal lineage of oocyte donors. *Biol Reprod* **66**: 367-373

Buczinski SM, Fecteau G, Lefebvre RC, Smith LC (2007) Fetal well-being assessment in bovine near-term gestations: current knowledge and future perspectives arising from comparative medicine. *Can Vet J* **48**: 178-183

Bui LC, Leandri RD, Renard JP, Duranthon V (2005) SSH adequacy to preimplantation mammalian development: scarce specific transcripts cloning despite irregular normalisation. *BMC Genomics* **6**: 155

Bulman DC, Lamming GE (1979) The use of milk progesterone analysis in the study of oestrus detection, herd fertility and embryonic mortality in dairy cows. *Br Vet J* **135**: 559-567

Burns K (2007) Animal clones in the food supply. *J Am Vet Med Assoc* **230**: 464-6, 468

Byers SL, Payson SJ, Taft RA (2006) Performance of ten inbred mouse strains following assisted reproductive technologies (ARTs). *Theriogenology* **65**: 1716-1726

Byrne JA, Simonsson S, Gurdon JB (2002) From intestine to muscle: nuclear reprogramming through defective cloned embryos. *Proc Natl Acad Sci U S A* **99**: 6059-6063

Camargo LS, Powell AM, Filho VR, Wall RJ (2005) Comparison of gene expression in individual preimplantation bovine embryos produced by in vitro fertilisation or somatic cell nuclear transfer. *Reprod Fertil Dev* **17**: 487-496

Cameron RDA (1999) Diseases of the Skin. In *Diseases of Swine*, Straw BE, D'Allaire S, Mengeling WL, Taylor DJ (eds) pp 954. Iowa State University Press: Ames

Campbell KH (2007) Ten years of cloning: questions answered and personal reflections. *Cloning Stem Cells* **9**: 8-11

Campbell KH, Fisher P, Chen WC, Choi I, Kelly RD, Lee JH, Xhu J (2007) Somatic cell nuclear transfer: Past, present and future perspectives. *Theriogenology*

Campbell KH, McWhir J, Ritchie WA, Wilmut I (1996) Sheep cloned by nuclear transfer from a cultured cell line. *Nature* **380**: 64-66

Caraviello DZ, Weigel KA, Fricke PM, Wiltbank MC, Florent MJ, Cook NB, Nordlund KV, Zwald NR, Rawson CL (2006) Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. *J Dairy Sci* **89**: 4723-4735

Carneiro G, Lorenzo P, Pimentel C, Pegoraro L, Bertolini M, Ball B, Anderson G, Liu I (2001) Influence of insulin-like growth factor-I and its interaction with gonadotropins, estradiol, and fetal calf serum on in vitro maturation and parthenogenic development in equine oocytes. *Biol Reprod* **65**: 899-905

Caroprese M, Albenzio M, Annicchiarico G, Sevi A (2006) Changes occurring in immune responsiveness of single- and twin-bearing Comisana ewes during the transition period. *J Dairy Sci* **89**: 562-568

Carroll JA, Carter DB, Korte SW, Prather RS (2005) Evaluation of the acute phase response in cloned pigs following a lipopolysaccharide challenge. *Domest Anim Endocrinol* **29**: 564-572

Carstens GE (1994) Cold thermoregulation in the newborn calf. *Vet Clin North Am Food Anim Pract* **10**: 69-106

Carstens GE, Glaser DE, Byers FM, Greene LW, Lunt DK (1997) Effects of bovine somatotropin treatment and intermittent growth pattern on mammary gland development in heifers. *J Anim Sci* **75**: 2378-2388

Carstens GE, Mostyn PM, Lammoglia MA, Vann RC, Apter RC, Randel RD (1997) Genotypic effects on norepinephrine-induced changes in thermogenesis, metabolic hormones, and metabolites in newborn calves. *J Anim Sci* **75**: 1746-1755

Carter DB, Lai L, Park KW, Samuel M, Lattimer JC, Jordan KR, Estes DM, Besch-Williford C, Prather RS (2002) Phenotyping of transgenic cloned piglets. *Cloning Stem Cells* **4**: 131-145

Casellas J, Caja G, Such X, Piedrafita J (2007) Survival analysis from birth to slaughter of Ripollesa lambs under semi-intensive management. *J Anim Sci* **85**: 512-517

Caulfield T, Bubela T (2007) Why a criminal ban? Analyzing the arguments against somatic cell nuclear transfer in the Canadian parliamentary debate. *Am J Bioeth* **7**: 51-61

Cavaleri F, Gentile L, Scholer HR, Boiani M (2006) Recombinant human albumin supports development of somatic cell nuclear transfer embryos in mice: toward the establishment of a chemically defined cloning protocol. *Cloning Stem Cells* **8**: 24-40

Cervera MT, Ruiz-Garcia L, Martinez-Zapater JM (2002) Analysis of DNA methylation in Arabidopsis thaliana based on methylation-sensitive AFLP markers. *Mol Genet Genomics* **268**: 543-552

Cesari A, Kaiser GG, Mucci N, Mutto A, Vincenti A, Fornes MW, Alberio RH (2006) Integrated morphophysiological assessment of two methods for sperm selection in bovine embryo production in vitro. *Theriogenology* **66**: 1185-1193

Cezar GG (2003) Epigenetic reprogramming of cloned animals. *Cloning Stem Cells* **5**: 165-180

Cezar GG, Bartolomei MS, Forsberg EJ, First NL, Bishop MD, Eilertsen KJ (2003) Genome-wide epigenetic alterations in cloned bovine fetuses. *Biol Reprod* **68**: 1009-1014

Chae JI, Cho SK, Seo JW, Yoon TS, Lee KS, Kim JH, Lee KK, Han YM, Yu K (2006) Proteomic analysis of the extraembryonic tissue from cloned porcine embryos. *Mol Cell Proteomics* **5**: 1559-1566

Chambers PA, Duggan PS, Heritage J, Forbes JM (2002) The fate of antibiotic resistance marker genes in transgenic plant feed material fed to chickens. *J Antimicrob Chemother* **49**: 161-164

CHANG MC (1959) Fertilization of rabbit ova in vitro. *Nature* **184(Suppl 7)**: 466-467

Chavatte-Palmer P, de SN, Laigre P, Camous S, Ponter AA, Beckers JF, Heyman Y (2006) Ultrasound fetal measurements and pregnancy associated glycoprotein secretion in early pregnancy in cattle recipients carrying somatic clones. *Theriogenology* **66**: 829-840

Chavatte-Palmer P, Heyman Y, Richard C, Monget P, LeBourhis D, Kann G, Chillard Y, Vignon X, Renard JP (2002) Clinical, hormonal, and hematologic characteristics of bovine calves derived from nuclei from somatic cells. *Biol Reprod* 1596-1603

Chavatte-Palmer P, Remy D, Cordonnier N, Richard C, Issenman H, Laigre P, Heyman Y, Mialot JP (2004) Health status of cloned cattle at different ages. *Cloning Stem Cells* **6**: 94-100

Chen DY, Jiang MX, Zhao ZJ, Wang HL, Sun QY, Zhang LS, Li RC, Cao HH, Zhang QJ, Ma DL (2007) Cloning of Asian yellow goat (C. hircus) by somatic cell nuclear transfer: telophase enucleation combined with whole cell intracytoplasmic injection. *Mol Reprod Dev* **74**: 28-34

Chen JQ, Pan Y, Zhao JY, Cao YY, Xu XJ, Zhou RJ, Zhang SL, Wang SY, Cheng GX (2002) [Study on the cloning goat by cross-breed somatic cell nuclear transfer]. *Shi Yan Sheng Wu Xue Bao* **35**: 278-282

Chen N, Liow SL, Yip WY, Tan LG, Tong GQ, Ng SC (2006) Early development of reconstructed embryos after somatic cell nuclear transfer in a non-human primate. *Theriogenology* **66**: 1300-1306

Chen T, Jiang Y, Zhang YL, Liu JH, Hou Y, Schatten H, Chen DY, Sun QY (2005) DNA hypomethylation of individual sequences in aborted cloned bovine fetuses. *Frontiers in Bioscience* **10**: 3002-3008

Chen T, Zhang YL, Jiang Y, Liu JH, Schatten H, Chen DY, Sun QY (2006) Interspecies nuclear transfer reveals that demethylation of specific repetitive sequences is determined by recipient ooplasm but not by donor intrinsic property in cloned embryos. *Mol Reprod Dev* **73**: 313-317

Cheng Y, Wang YG, Luo JP, Shen Y, Yang YF, Ju HM, Zou XG, Xu SF, Lao WD, Du M (2002) [Cloned goats produced from the somatic cells of an adult transgenic goat]. *Sheng Wu Gong Cheng Xue Bao* **18**: 79-83

Chesne P, Adenot PG, Viglietta C, Baratte M, Boulanger L, Renard JP (2002) Cloned rabbits produced by nuclear transfer from adult somatic cells. *Nat Biotechnol* **20**: 366-369

Cheung P, Lau P (2005) Epigenetic regulation by histone methylation and histone variants. *Mol Endocrinol* **19**: 563-573

Cho J, Bhuiyan MM, Shin S, Park E, Jang G, Kang S, Lee B, Hwang W (2004) Development potential of transgenic somatic cell nuclear transfer embryos according to various factors of donor cell. *J Vet Med Sci* **66**: 1567-1573

Choi YH, Chung YG, Walker SC, Westhusin ME, Hinrichs K (2003) In vitro development of equine nuclear transfer embryos: effects of oocyte maturation media and amino acid composition during embryo culture. *Zygote* **11**: 77-86

Choi YH, Love CC, Chung YG, Varner DD, Westhusin ME, Burghardt RC, Hinrichs K (2002) Production of nuclear transfer horse embryos by Piezo-driven injection of somatic cell nuclei and activation with stallion sperm cytosolic extract. *Biol Reprod* **67**: 561-567

Choi YH, Love LB, Westhusin ME, Hinrichs K (2003) Activation of equine nuclear transfer oocytes: methods and timing of treatment in relation to nuclear remodeling. *Biol Reprod* **70** : 46-53

Christley RM, Morgan KL, Parkin TD, French NP (2003) Factors related to the risk of neonatal mortality, birth-weight and serum immunoglobulin concentration in lambs in the UK. *Prev Vet Med* **57**: 209-226

Chung YG, Gao S, Latham KE (2006) Optimization of procedures for cloning by somatic cell nuclear transfer in mice. *Methods Mol Biol* **348**: 111-124

Chung YG, Ratnam S, Chaillet JR, Latham KE (2003) Abnormal regulation of DNA methyltransferase expression in cloned mouse embryos. *Biol Reprod* **69**: 146-153

Church SL (2006) Nuclear transfer saddles up. *Nat Biotechnol* **24**: 605-607

Cibelli JB, Campbell KH, Seidel GE, West MD, Lanza RP (2002) The health profile of cloned animals. *Nat Biotechnol* **20**: 13-14

Cibelli JB, Stice SL, Golueke PJ, Kane JJ, Jerry J, Blackwell C, Ponce de Leon FA, Robl JM (1998) Cloned transgenic calves produced from nonquiescent fetal fibroblasts. *Science* **280**: 1256-1258

Clark AJ, Ferrier P, Aslam S, Burl S, Denning C, Wylie D, Ross A, de SP, Wilmut I, Cui W (2003) Proliferative lifespan is conserved after nuclear transfer. *Nat Cell Biol* **5**: 535-538

Coghlan A (2004) Cloners press on despite UK nay. *New Sci* **182**: 9

Cole JB, Wiggans GR, VanRaden PM (2007) Genetic evaluation of stillbirth in United States Holsteins using a sire-maternal grandsire threshold model. *J Dairy Sci* **90**: 2480-2488

Cole JB, Wiggans GR, VanRaden PM, Miller RH (2007) Stillbirth (co)variance components for a sire-maternal grandsire threshold model and development of a calving ability index for sire selection. *J Dairy Sci* **90**: 2489-2496

Collas P, Taranger CK (2006) Toward reprogramming cells to pluripotency. *Ernst Schering Res Found Workshop* 47-67

Collas P, Taranger CK, Boquest AC, Noer A, Dahl JA (2006) On the way to reprogramming cells to pluripotency using cell-free extracts. *Reprod Biomed Online* **12**: 762-770

Collier RJ, Beede DK, Thatcher WW, Israel LA, Wilcox CJ (1982) Influences of environment and its modification on dairy animal health and production. *J Dairy Sci* **65**: 2213-2227

Colman A (2002) Turning back the developmental clock. *Nat Biotechnol* **20**: 348-349

Committee to review the OMB risk assessment bulletin (2007) *Scientific reveiw of the proposed risk assessment bulletin from the Office of Management and Budget*. National Research Council:

Constant F, Guillomot M, Heyman Y, Vignon X, Laigre P, Servely JL, Renard JP, Chavatte-Palmer P (2006) Large offspring or large placenta syndrome? Morphometric analysis of late gestation bovine placentomes from somatic nuclear transfer pregnancies complicated by hydrallantois. *Biol Reprod* **75**: 122-130

Cooney CA, Dave AA, Wolff GL (2002) Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. *J Nutr* **132**: 2393S-2400S

Croney CC, Millman ST (2007) Board-invited review: the ethical and behavioral bases for farm animal welfare legislation. *J Anim Sci* **85**: 556-565

Cropley JE, Suter CM, Beckman KB, Martin DI (2006) Germ-line epigenetic modification of the murine A vy allele by nutritional supplementation. *Proc Natl Acad Sci U S A* **103**: 17308-17312

Crosier AE, Farin CE, Rodriguez KF, Blondin P, Alexander JE, Farin PW (2002) Development of skeletal muscle and expression of candidate genes in bovine fetuses from embryos produced in vivo or in vitro. *Biol Reprod* **67**: 401-408

Cyagra. Cyagra IETS Poster. 2004.
Ref Type: Conference Proceeding

Dai Y, Wang L, Wang H, Liu Y, Li N, Lyu Q, Keefe DL, Albertini DF, Liu L (2006) Fate of centrosomes following somatic cell nuclear transfer (SCNT) in bovine oocytes. *Reproduction* **131**: 1051-1061

Daniels R, Hall V, Trounson AO (2000) Analysis of gene transcription in bovine nuclear transfer embryos reconstructed with granulosa cell nuclei. *Biol Reprod* **63**: 1034-1040

Davidson EH, McClay DR, Hood L (2003) Regulatory gene networks and the properties of the developmental process. *Proc Natl Acad Sci U S A* **100**: 1475-1480

Davies CJ (2007) Why is the fetal allograft not rejected? *J Anim Sci* **85**: E32-E35

Davies CJ, Hill JR, Edwards JL, Schrick FN, Fisher PJ, Eldridge JA, Schlafer DH (2004) Major histocompatibility antigen expression on the bovine placenta: its relationship to abnormal pregnancies and retained placenta. *Anim Reprod Sci* **82-83**: 267-280

Davis ME, Simmen RC (2000) Genetic parameter estimates for serum insulin-like growth factor-I concentration and carcass traits in Angus beef cattle. *J Anim Sci* **78**: 2305-2313

Davis TL, Yang GJ, McCarrey JR, Bartolomei MS (2000) The H19 methylation imprint is erased and re-established differentially on the parental alleles during male germ cell development. *Hum Mol Genet* **9**: 2885-2894

De La Torre-Sanchez JF, Preis K, Seidel GE (2006) Metabolic regulation of in-vitro-produced bovine embryos. I. Effects of metabolic regulators at different glucose concentrations with embryos produced by semen from different bulls. *Reprod Fertil Dev* **18**: 585-596

de Montera B, Boulanger L, Taourit S, Renard JP, Eggen A (2004) Genetic identity of clones and methods to explore DNA. *Cloning Stem Cells* **6**: 133-139

De Sousa PA, Dobrinsky JR, Zhu J, Archibald AL, Ainslie A, Bosma W, Bowering J, Bracken J, Ferrier PM, Fletcher J, Gasparrini B, Harkness L, Johnston P, Ritchie M, Ritchie WA, Travers A, Albertini D, Dinnyes A, King TJ, Wilmut I (2002) Somatic cell nuclear transfer in the pig: control of pronuclear formation and integration with improved methods for activation and maintenance of pregnancy. *Biol Reprod* **66**: 642-650

De Sousa PA, King T, Harkness L, Young LE, Walker SK, Wilmut I (2001) Evaluation of gestational deficiencies in cloned sheep fetuses and placentae. *Biol Reprod* **65**: 23-30

De Sousa PA, Winger Q, Hill JR, Jones K, Watson AJ, Westhusin ME (1999) Reprogramming of fibroblast nuclei after transfer into bovine oocytes. *Cloning* **1**: 63-69

Dean W, Santos F, Reik W (2003) Epigenetic reprogramming in early mammalian development and following somatic nuclear transfer. *Semin Cell Dev Biol* **14**: 93-100

Dean W, Santos F, Stojkovic M, Zakhartchenko V, Walter J, Wolf E, Reik W (2001) Conservation of methylation reprogramming in mammalian development: aberrant reprogramming in cloned embryos. *Proc Natl Acad Sci U S A* **98**: 13734-13738

Denning C, Burl S, Ainslie A, Bracken J, Dinnyes A, Fletcher J, King T, Ritchie M, Ritchie WA, Rollo M, De Sousa P, Travers A, Wilmut I, Clark AJ (2001) Deletion of the alpha(1,3)galactosyl transferase (GGTA1) gene and the prion protein (PrP) gene in sheep. *Nat Biotechnol* **19**: 559-562

DeRouchey JM, Dritz SS, Goodband RD, Nelssen JL, and Tokach MD. Kansas Stae University Swine Nutrition Guide: General nutrition principles for swine. 2007. Manhattan, KS, Kansas State University Agricultural Experiment Station and Cooperative Extension Service.
Ref Type: Pamphlet

Diles JJB, Green RD, Shepard HH, Hughes LJ, Mathiews GL (1996) Variation Within and Between Groups of Genetically Identical Nuclear Transfer Brangus Bulls and Steers for Growth Measures Taken Over Time. *The Professional Animal Scientist* **12**: 250-256

Diles JJB, Green RD, Shepard HH, Mathiews GL, Hughes LJ, Miller MF (1996) Relationships Between Body Measurements Obtained on Yearling Brangus Bulls and Measures of Carcass Merit Obtained from Their Steer Clone-Mates. *The Professional Animal Scientist* **12**: 244-249

Dindot SV, Farin PW, Farin CE, Romano J, Walker S, Long C, Piedrahita JA (2004) Epigenetic and genomic imprinting analysis in nuclear transfer derived Bos gaurus/Bos taurus hybrid fetuses. *Biol Reprod* **71**: 470-478

Dinnyes A, Dai Y, Jiang S, Yang X (2000) High developmental rates of vitrified bovine oocytes following parthenogenetic activation, in vitro fertilization, and somatic cell nuclear transfer. *Biol Reprod* **63**: 513-518

Dong YJ, Bai XJ, Li JD, Suzuki T (2003) [Isolation and nuclear transfer of ES-like cells colonies derived from embryos being cloning of bovine somatic]. *Yi Chuan Xue Bao* **30**: 114-118

Donovan GA (1992) Management of Cow and Newborn Calf at Calving. In *Large Dairy Herd Management*, Van Horn HH, Wilcox CJ (eds) pp 393-400. American Dairy Science Association: Champaigne

Drost M, Ambrose JD, Thatcher MJ, Cantrell CK, Wolfsdorf KE, Hasler JF, Thatcher WW (1999) Conception rates after artificial insemination or embryo transfer in lactating dairy cows during summer in Florida. *Theriogenology* **52**: 1161-1167

Drouet M, Boutet S, Lauret MG, Chene J, Bonneau JC, Le SJ, Hassoun S, Gay G, Sabbah A (1994) [The pork-cat syndrome or crossed allergy between pork meat and cat epithelia (1)]. *Allerg Immunol (Paris)* **26**: 166-2

Du F, Shen Pc, Xu J, Sung LY, Jeong BS, Lucky Nedambale T, Riesen J, Cindy Tian X, Cheng WTK, Lee SN, Yang X (2006) The cell agglutination agent, phytohemagglutinin-L, improves the efficiency of somatic nuclear transfer cloning in cattle (Bos taurus). *Theriogenology* **65**: 642-657

Du F, Shen PC, Xu J, Sung LY, Jeong BS, Lucky NT, Riesen J, Cindy T, X, Cheng WT, Lee SN, Yang X (2005) The cell agglutination agent, phytohemagglutinin-L, improves the efficiency of somatic nuclear transfer cloning in cattle (Bos taurus). *Theriogenology*

Du Y, Zhang Y, Li J, Kragh PM, Kuwayama M, Ieda S, Zhang X, Schmidt M, Bogh IB, Purup S, Pedersen AM, Villemoes K, Yang H, Bolund L, Vajta G (2007) Simplified cryopreservation of porcine cloned blastocysts. *Cryobiology* **54**: 181-187

Du Z, Zhao D, Zhao Y, Wang S, Gao Y, Li N (2007) Identification and characterization of bovine regulator of telomere length elongation helicase gene (RTEL): molecular cloning, expression distribution, splice variants and DNA methylation profile. *BMC Mol Biol* **8**: 18

Duncan JR, Prasse KW (1986) *Veterinary Laboratory Medicine Clinical Pathology*. Iowa State University Press: Ames

Duncan JR, Prasse KW (2003) *Veterinary Laboratory Medicine Clinical Pathology*. Iowa State University Press: Ames

Dunne LD, Diskin MG, Sreenan JM (2000) Embryo and foetal loss in beef heifers between day 14 of gestation and full term. *Anim Reprod Sci* **58**: 39-44

Dwyer CM (2003) Behavioural development in the neonatal lamb: effect of maternal and birth-related factors. *Theriogenology* **59**: 1027-1050

Dwyer CM, Morgan CA (2006) Maintenance of body temperature in the neonatal lamb: effects of breed, birth weight, and litter size. *J Anim Sci* **84**: 1093-1101

Dyer O (2002) Dolly's arthritis dents faith in cloning. *BMJ* **324**: 67

Dziuk P (1985) Effect of migration, distribution and spacing of pig embryos on pregnancy and fetal survival. *J Reprod Fertil Suppl* **33**: 57-63

Edwards JL, Schrick FN, McCracken MD, van Amstel SR, Hopkins FM, Welborn MG, Davies CJ (2003) Cloning adult farm animals: a review of the possibilities and problems associated with somatic cell nuclear transfer. *Am J Reprod Immunol* **50**: 113-123

Edwards L, Peura T, Hartwich K, Rudiger S, McMillen IC, Walker S (2002) Postnatal growth and circulating ACTH and cortisol concentrations during the first month of life in cloned lambs. *Endocrinology* **143**: 3699-3702

Eggan K, Akutsu H, Loring J, Jackson-Grusby L, Klemm M, Rideout WM, III, Yanagimachi R, Jaenisch R (2001) Hybrid vigor, fetal overgrowth, and viability of mice derived by nuclear cloning and tetraploid embryo complementation. *Proc Natl Acad Sci U S A* **98**: 6209-6214

Egger G, Liang G, Aparicio A, Jones PA (2004) Epigenetics in human disease and prospects for epigenetic therapy. *Nature* **429**: 457-463

Eilertsen KJ, Power RA, Harkins LL, Misica P (2007) Targeting cellular memory to reprogram the epigenome, restore potential, and improve somatic cell nuclear transfer. *Anim Reprod Sci* **98**: 129-146

Ellis SA (2004) Immune status: normal expression of MHC class I in the placenta and what is expected in clones. *Cloning Stem Cells* **6**: 121-125

Elsasser TH, Rumsey TS, Hammond AC (1989) Influence of diet on basal and growth hormone-stimulated plasma concentrations of IGF-I in beef cattle. *J Anim Sci* **67**: 128-141

El-Zarkouny SZ, Cartmill JA, Hensley BA, Stevenson JS (2004) Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone. *J Dairy Sci* **87**: 1024-1037

Engeland IV, Ropstad E, Kindahl H, Andresen O, Waldeland H, Tverdal A (1999) Foetal loss in dairy goats: function of the adrenal glands, corpus luteum and the foetal-placental unit. *Anim Reprod Sci* **55**: 205-222

Engeland IV, Waldeland H, Andresen O, Tverdal A (1997) Foetal loss in dairy goats: an epidemiological study in 515 individual goats. *Anim Reprod Sci* **49**: 45-53

Enright BP, Sung LY, Chang CC, Yang X, Tian XC (2005) Methylation and acetylation characteristics of cloned bovine embryos from donor cells treated with 5-aza-2'-deoxycytidine. *Biol Reprod* **72**: 944-948

Enright BP, Taneja M, Schreiber D, Riesen J, Tian XC, Fortune JE, Yang X (2002) Reproductive characteristics of cloned heifers derived from adult somatic cells. *Biol Reprod* **66**: 291-296

Espejo LA, Endres MI, Salfer JA (2006) Prevalence of lameness in high-producing holstein cows housed in freestall barns in Minnesota. *J Dairy Sci* **89**: 3052-3058

Estrada J, Sommer J, Collins B, Mir B, Martin A, York A, Petters RM, Piedrahita JA (2007) Swine generated by somatic cell nuclear transfer have increased incidence of intrauterine growth restriction (IUGR). *Cloning Stem Cells* **9**: 229-236

Evans MJ, Gurer C, Loike JD, Wilmut I, Schnieke AE, Schon EA (1999) Mitochondrial DNA genotypes in nuclear transfer-derived cloned sheep. *Nat Genet* **23**: 90-93

Even MS, Sandusky CB, Barnard ND (2006) Serum-free hybridoma culture: ethical, scientific and safety considerations. *Trends Biotechnol* **24**: 105-108

Exl BM, Fritsche R (2001) Cow's milk protein allergy and possible means for its prevention. *Nutrition* **17**: 642-651

Faast R, Harrison SJ, Beebe LF, McIlfatrick SM, Ashman RJ, Nottle MB (2006) Use of adult mesenchymal stem cells isolated from bone marrow and blood for somatic cell nuclear transfer in pigs. *Cloning Stem Cells* **8**: 166-173

Faber DC, Ferre LB, Metzger J, Robl JM, Kasinathan P (2004) Agro-economic impact of cattle cloning. *Cloning Stem Cells* **6**: 198-207

Faber, D. and ferre LB. Advancements in reproductive technology in cattle. Not Listed . 2004. Ref Type: Abstract

Fahrudin M, Otoi T, Karja NW, Mori M, Murakami M, Suzuki T (2002) Analysis of DNA fragmentation in bovine somatic nuclear transfer embryos using TUNEL. *Reproduction* **124**: 813-819

Fang ZF, Gai H, Huang YZ, Li SG, Chen XJ, Shi JJ, Wu L, Liu A, Xu P, Sheng HZ (2006) Rabbit embryonic stem cell lines derived from fertilized, parthenogenetic or somatic cell nuclear transfer embryos. *Exp Cell Res* **312**: 3669-3682

Farin CE, Farin PW, Piedrahita JA (2004) Development of fetuses from in vitro-produced and cloned bovine embryos. *J Anim Sci* **82 E-Suppl**: E53-E62

Farin PW, Crosier AE, Farin CE (2001) Influence of in vitro systems on embryo survival and fetal development in cattle. *Theriogenology* **55**: 151-170

Farin PW, Farin CE (1995) Transfer of bovine embryos produced in vivo or in vitro: survival and fetal development. *Biol Reprod* **52**: 676-682

Farin PW, Piedrahita JA, Farin CE (2006) Errors in development of fetuses and placentas from in vitro-produced bovine embryos. *Theriogenology* **65**: 178-191

Fazzari MJ, Greally JM (2004) Epigenomics: beyond CpG islands. *Nat Rev Genet* **5**: 446-455

Fecteau ME, Palmer JE, Wilkins PA (2005) Neonatal care of high-risk cloned and transgenic calves. *Vet Clin North Am Food Anim Pract* **21**: 637-53, vi

Fiocchi A, Restani P, Riva E (2000) Beef allergy in children. *Nutrition* **16**: 454-457

First NL, Prather RS (1991) Production of embryos by oocyte cytoplast-blastomere fusion in domestic animals. *J Reprod Fertil Suppl* **43**: 245-254

First NL, Sims MM, Park SP, Kent-First MJ (1994) Systems for production of calves from cultured bovine embryonic cells. *Reprod Fertil Dev* **6**: 553-562

Fischer-Brown AE, Lindsey BR, Ireland FA, Northey DL, Monson RL, Clark SG, Wheeler MB, Kesler DJ, Lane SJ, Weigel KA, Rutledge JJ (2005) Embryonic disc development and subsequent viability of cattle embryos following culture in two media under two oxygen concentrations. *Reprod Fertil Dev* **16**: 787-793

Flechon JE (2006) Analysis of the nucleolar compartment of the nucleus as an indicator of nuclear reprogramming after nuclear transfer. *Methods Mol Biol* **325**: 225-238

Fletcher CJ, Roberts CT, Hartwich KM, Walker SK, McMillen IC (2007) Somatic cell nuclear transfer in the sheep induces placental defects that likely precede fetal demise. *Reproduction* **133**: 243-255

Flis SA, Wattiaux MA (2005) Effects of parity and supply of rumen-degraded and undegraded protein on production and nitrogen balance in Holsteins. *J Dairy Sci* **88**: 2096-2106

Foote RH (2001) Inhibition of sperm motility does not affect live-dead separation of bull sperm by glass beads. *Asian J Androl* **3**: 193-198

Forsberg EJ, Strelchenko NS, Augenstein ML, Betthauser JM, Childs LA, Eilertsen KJ, Enos JM, Forsythe TM, Golueke PJ, Koppang RW, Lange G, Lesmeister TL, Mallon KS, Mell GD, Misica PM, Pace MM, Pfister-Genskow M, Voelker GR, Watt SR, Bishop MD (2002) Production of cloned cattle from in vitro systems. *Biol Reprod* **67**: 327-333

Forsyth JT, Wells DN (2006) Health and neonatal care of bovine clones. *Methods Mol Biol* **348**: 91-108

Foster KA, Colditz PB, Lingwood BE, Burke C, Dunster KR, Roberts MS (2001) An improved survival model of hypoxia/ischaemia in the piglet suitable for neuroprotection studies. *Brain Res* **919**: 122-131

Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suner D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu YZ, Plass C, Esteller M (2005) Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci U S A*

Franco M, Block J, Jousan FD, de Castro e Paula, Brad AM, Franco JM, Grisel F, Monson RL, Rutledge JJ, Hansen PJ (2006) Effect of transfer of one or two in vitro-produced embryos and post-transfer administration of gonadotropin releasing hormone on pregnancy rates of heat-stressed dairy cattle. *Theriogenology* **66**: 224-233

Fujimori T, Kurotaki Y, Miyazaki J, Nabeshima Y (2003) Analysis of cell lineage in two- and four-cell mouse embryos. *Development* **130**: 5113-5122

Fuks F (2005) DNA methylation and histone modifications: teaming up to silence genes. *Curr Opin Genet Dev*

Fulka J, Jr., Fulka H (2007) Somatic cell nuclear transfer (SCNT) in mammals: the cytoplast and its reprogramming activities. *Adv Exp Med Biol* **591**: 93-102

Fulka J, Jr., Loi P, Ledda S, Moor RM, Fulka J (2001) Nucleus transfer in mammals: how the oocyte cytoplasm modifies the transferred nucleus. *Theriogenology* **55**: 1373-1380

Fulka J, Jr., Miyashita N, Nagai T, Ogura A (2004) Do cloned mammals skip a reprogramming step? *Nat Biotechnol* **22**: 25-26

Galli C, Colleoni S, Duchi R, Lagutina I, Lazzari G (2007) Developmental competence of equine oocytes and embryos obtained by in vitro procedures ranging from in vitro maturation and ICSI to embryo culture, cryopreservation and somatic cell nuclear transfer. *Anim Reprod Sci* **98**: 39-55

Galli C, Duchi R, Crotti G, Turini P, Ponderato N, Colleoni S, Lagutina I, Lazzari G (2003) Bovine embryo technologies. *Theriogenology* **59**: 599-616

Galli C, Lagutina I, Crotti G, Colleoni S, Turini P, Ponderato N, Duchi R, Lazzari G (2003) Pregnancy: a cloned horse born to its dam twin. *Nature* **424**: 635

Galli C, Lagutina I, Lazzari G (2003) Introduction to cloning by nuclear transplantation. *Cloning Stem Cells* **5**: 223-232

Galli C, Lagutina I, Vassiliev I, Duchi R, Lazzari G (2002) Comparison of microinjection (piezo-electric) and cell fusion for nuclear transfer success with different cell types in cattle. *Cloning Stem Cells* **4**: 189-196

Galli C, Vassiliev I, Lagutina I, Galli A, Lazzari G (2003) Bovine embryo development following ICSI: effect of activation, sperm capacitation and pre-treatment with dithiothreitol. *Theriogenology* **60**: 1467-1480

Galli, C., Duchi, R., Moor, R. M., and Lazzari, G. Mammalian Leukocytes Contain All the Genetic Information Necessary for the Development of New Individual. 161-170. 1999.
Ref Type: Generic

Gardner DK, Lane M (2005) Ex vivo early embryo development and effects on gene expression and imprinting. *Reprod Fertil Dev* **17**: 361-370

Garry FB, Adams R, McCann JP, Odde KG (1996) Postnatal characteristics of calves produced by nuclear transfer cloning. *Theriogenology* **45**: 141-152

Gatphayak, K, Chongkasikit, N, Charoensook, R, Laenoi, W, Vearasilp, T, Sardsud, V, Knorr, C, ter Meulen, U, and Brenig, B. Present Situation of Porcine Hernia inguinalis/scrotalis in Thailand. 10-11-2005. 10-11-2005.
Ref Type: Conference Proceeding

Gauthier M, Pierson J, Drolet M, Bhatia B, Baldassarre H, Keefer CL (2001) Sexual maturation and fertility of male Nigerian Dwarf goat (Capra hircus) clones produced by somatic cell nuclear transfer. *Cloning Stem Cells* **3**: 151-155

Gibbons J, Arat S, Rzucidlo J, Miyoshi K, Waltenburg R, Respess D, Venable A, Stice S (2002) Enhanced survivability of cloned calves derived from roscovitine-treated adult somatic cells. *Biol Reprod* **66**: 895-900

Giraldo AM, Lynn JW, Godke RA, Bondioli KR (2006) Proliferative characteristics and chromosomal stability of bovine donor cells for nuclear transfer. *Mol Reprod Dev* **73**: 1230-1238

Giraldo AM, Lynn JW, Purpera MN, Godke RA, Bondioli KR (2007) DNA methylation and histone acetylation patterns in cultured bovine fibroblasts for nuclear transfer. *Mol Reprod Dev*

Gjerris M and Vatja G. The science and technology of farm animal cloning. Tveit g and Sandoe P. 2005. Frederiksberg, Danish centre for bioethics and risk assessment.
Ref Type: Report

Gjorret JO, Wengle J, Maddox-Hyttel P, King WA (2005) Chronological appearance of apoptosis in bovine embryos reconstructed by somatic cell nuclear transfer from quiescent granulosa cells. *Reprod Domest Anim* **40**: 210-216

Gomez MC, Jenkins JA, Giraldo A, Harris RF, King A, Dresser BL, Pope CE (2003) Nuclear transfer of synchronized african wild cat somatic cells into enucleated domestic cat oocytes. *Biol Reprod* **69**: 1032-1041

Gong G, Dai Y, Fan B, Zhu H, Zhu S, Wang H, Wang L, Tang B, Li R, Wan R, Liu Y, Huang Y, Zhang L, Sun X, Li N (2004) Birth of calves expressing the enhanced green fluorescent protein after transfer of fresh or vitrified/thawed blastocysts produced by somatic cell nuclear transfer. *Mol Reprod Dev* **69**: 278-288

Gong G, Dai Y, Zhu H, Wang H, Wang L, Li R, Wan R, Liu Y, Li N (2004) Generation of cloned calves from different types of somatic cells. *Sci China C Life Sci* **47**: 470-476

Goodhand KL, Watt RG, Staines ME, Hutchinson JS, Broadbent PJ (1999) In vivo oocyte recovery and in vitro embryo production from bovine donors aspirated at different frequencies or following FSH treatment. *Theriogenology* **51**: 951-961

Gotoh K, Inoue K, Ogura A, Oishi M (2006) Intra-strain polymorphisms are detected but no genomic alteration is found in cloned mice. *Biochem Biophys Res Commun* **348**: 166-169

Govoni KE, Tian XC, Kazmer GW, Taneja M, Enright BP, Rivard AL, Yang X, Zinn SA (2002) Age-related changes of the somatotropic axis in cloned Holstein calves. *Biol Reprod* **66**: 1293-1298

Green AL, Wells DN, Oback B (2007) Cattle Cloned from Increasingly Differentiated Muscle Cells. *Biol Reprod*

Greve T, Callesen H (2005) Embryo technology: implications for fertility in cattle. *Rev Sci Tech* **24**: 405-412

Gupta MK, Uhm SJ, Han DW, Lee HT (2007) Embryo quality and production efficiency of porcine parthenotes is improved by phytohemagglutinin. *Mol Reprod Dev* **74**: 435-444

Gurdon JB, Uehlinger V (1966) "Fertile" intestine nuclei. *Nature* **210**: 1240-1241

Hadley GL, Wolf CA, Harsh SB (2006) Dairy cattle culling patterns, explanations, and implications. *J Dairy Sci* **89**: 2286-2296

Hafez B, Hafez ESE (2000) *Reproduction in farm animals*. Lippincott Williams & Wilkins: Philadelphia

Hajkova P, Erhardt S, Lane N, Haaf T, El Maarri O, Reik W, Walter J, Surani MA (2002) Epigenetic reprogramming in mouse primordial germ cells. *Mech Dev* **117**: 15-23

Hales CN, Barker DJ (2001) The thrifty phenotype hypothesis. *Br Med Bull* **60**: 5-20

Hall VJ, Cooney MA, Shanahan P, Tecirlioglu RT, Ruddock NT, French AJ (2005) Nuclear lamin antigen and messenger RNA expression in bovine in vitro produced and nuclear transfer embryos. *Mol Reprod Dev* **72**: 471-482

Hall VJ, Ruddock NT, Cooney MA, Korfiatis NA, Tecirlioglu RT, Downie S, Williamson M, French AJ (2006) Production of a cloned calf using zona-free serial nuclear transfer. *Theriogenology* **65**: 424-440

Hall VJ, Ruddock NT, French AJ (2005) Expression profiling of genes crucial for placental and preimplantation development in bovine in vivo, in vitro, and nuclear transfer blastocysts. *Mol Reprod Dev*

Hamilton HM, Peura TT, Laurincik J, Walker SK, Maddocks S, Maddox-Hyttel P (2004) Ovine ooplasm directs initial nucleolar assembly in embryos cloned from ovine, bovine, and porcine cells. *Mol Reprod Dev* **69**: 117-125

Han GD, Matsuno M, Ito G, Ikeucht Y, Suzuki A (2000) Meat allergy: investigation of potential allergenic proteins in beef. *Biosci Biotechnol Biochem* **64**: 1887-1895

Han YM, Kang YK, Koo DB, Lee KK (2003) Nuclear reprogramming of cloned embryos produced in vitro. *Theriogenology* **59**: 33-44

Han ZM, Chen DY, Li JS, Sun QY, Wan QH, Kou ZH, Rao G, Lei L, Liu ZH, Fang SG (2004) Mitochondrial DNA heteroplasmy in calves cloned by using adult somatic cell. *Mol Reprod Dev* **67**: 207-214

Hanada H, Takeda K, Tagami T, Nirasawa K, Akagi S, Adachi N, Takahashi S, Izaike Y, Iwamoto M, Fuchimoto D, Miyashita N, Kubo M, Onishi A, King WA (2005) Chromosomal instability in the cattle clones derived by somatic cell nuclear-transfer. *Mol Reprod Dev* **71** : 36-44

Hare E, Norman HD, Wright JR (2006) Survival rates and productive herd life of dairy cattle in the United States. *J Dairy Sci* **89**: 3713-3720

Hart IC, Bines JA, Balch CC, Cowie AT (1975) Hormone and metabolite differences between lactating beef and dairy cattle. *Life Sci* **16**: 1285-1292

Hashizume K, Ishiwata H, Kizaki K, Yamada O, Takahashi T, Imai K, Patel OV, Akagi S, Shimizu M, Takahashi S, Katsuma S, Shiojima S, Hirasawa A, Tsujimoto G, Todoroki J, Izaike Y (2002) Implantation and placental development in somatic cell clone recipient cows. *Cloning Stem Cells* **4**: 197-209

Hasler JF (1987) Effect of donor-embryo-recipient pregnancy rate in a large-scale transfer program. *Theriogenology* **27**: 139-168

Hasler JF (2000) In vitro culture of bovine embryos in Menezo's B2 medium with or without coculture and serum: the normalcy of pregnancies and calves resulting from transferred embryos. *Anim Reprod Sci* **60-61**: 81-91

Hasler JF, Henderson WB, Hurtgen PJ, Jin ZQ, McCauley AD, Mower SA, Neely B, Shuey LS, Stokes JE, Trimmer SA (1995) Production, Freezing and Transfer of bovine IVF embryos and subsequent calving results. *Theriogenology* **43**: 141-152

Hayes O, Rodriguez LL, Gonzalez A, Falcon V, Aguilar A, Castro FO (2005) Effect of cryopreservation on fusion efficiency and in vitro development into blastocysts of bovine cell lines used in somatic cell cloning. *Zygote* **13**: 277-282

Heins BJ, Hansen LB, Seykora AJ (2006) Calving difficulty and stillbirths of pure Holsteins versus crossbreds of Holstein with Normande, Montbeliarde, and Scandinavian Red. *J Dairy Sci* **89**: 2805-2810

Heng BC, Cao T, Stojkovic M, Vajta G (2006) Mammalian oocyte polarity can be exploited for the automation of somatic cell nuclear transfer--in the development of a 'cloning biochip'. *Med Hypotheses* **67**: 420-421

Henney SR, Killian GJ, Deaver DR (1990) Libido, hormone concentrations in blood plasma and semen characteristics in Holstein bulls. *J Anim Sci* **68**: 2784-2792

Herath CB, Ishiwata H, Shiojima S, Kadowaki T, Katsuma S, Ushizawa K, Imai K, Takahashi T, Hirasawa A, Takahashi S, Izaike Y, Tsujimoto G, Hashizume K (2006) Developmental aberrations of liver gene expression in bovine fetuses derived from somatic cell nuclear transplantation. *Cloning Stem Cells* **8**: 79-95

Hernandez-Fonseca HJ, Sirisathien S, Bosch P, Cho HS, Lott JD, Hawkins LL, Hollett RB, Coley SL, Brackett BG (2002) Offspring resulting from direct transfer of cryopreserved bovine embryos produced in vitro in chemically defined media. *Anim Reprod Sci* **69**: 151-158

Hernandez-Mendo O, von Keyserlingk MA, Veira DM, Weary DM (2007) Effects of pasture on lameness in dairy cows. *J Dairy Sci* **90**: 1209-1214

Hessel EF, Reiners K, Van den Weghe HF (2006) Socializing piglets before weaning: effects on behavior of lactating sows, pre- and postweaning behavior, and performance of piglets. *J Anim Sci* **84**: 2847-2855

Heyman Y (2005) Nuclear transfer: a new tool for reproductive biotechnology in cattle. *Reprod Nutr Dev* **45**: 353-361

Heyman Y, Chavatte-Palmer P, Berthelot V, Fromentin G, Hocquette JF, Martignat L, Renard JP (2007) Assessing the quality of products from cloned cattle: an integrative approach. *Theriogenology* **67**: 134-141

Heyman Y, Chavatte-Palmer P, LeBourhis D, Camous S, Vignon X, Renard JP (2002) Frequency and occurrence of late-gestation losses from cattle cloned embryos. *Biol Reprod* **66**: 6-13

Heyman Y, Richard C, Rodriguez-Martinez H, Lazzari G, Chavatte-Palmer P, Vignon X, Galli C (2004) Zootechnical performance of cloned cattle and offspring: preliminary results. *Cloning Stem Cells* **6**: 111-120

Heyman Y, Zhou Q, LeBourhis D, Chavatte-Palmer P, Renard JP, Vignon X (2002) Novel approaches and hurdles to somatic cloning in cattle. *Cloning Stem Cells* **4**: 47-55

Hiendleder S (2007) Mitochondrial DNA inheritance after SCNT. *Adv Exp Med Biol* **591**: 103-116

Hiendleder S, Mund C, Reichenbach HD, Wenigerkind H, Brem G, Zakhartchenko V, Lyko F, Wolf E (2004) Tissue-specific elevated genomic cytosine methylation levels are associated with an overgrowth phenotype of bovine fetuses derived by in vitro techniques. *Biol Reprod* **71**: 217-223

Hiendleder S, Prelle K, Bruggerhoff K, Reichenbach HD, Wenigerkind H, Bebbere D, Stojkovic M, Muller S, Brem G, Zakhartchenko V, Wolf E (2004) Nuclear-cytoplasmic interactions affect in utero developmental capacity, phenotype, and cellular metabolism of bovine nuclear transfer fetuses. *Biol Reprod* **70**: 1196-1205

Hiendleder S, Wirtz M, Mund C, Klempt M, Reichenbach HD, Stojkovic M, Weppert M, Wenigerkind H, Elmlinger M, Lyko F, Schmitz OJ, Wolf E (2006) Tissue-specific effects of in vitro fertilization procedures on genomic cytosine methylation levels in overgrown and normal sized bovine fetuses. *Biol Reprod* **75**: 17-23

Higdon HL, III, Spitzer JC, Hopkins FM, Bridges WC, Jr. (2000) Outcomes of breeding soundness evaluation of 2898 yearling bulls subjected to different classification systems. *Theriogenology* **53**: 1321-1332

Hilger C, Kohnen M, Grigioni F, Lehners C, Hentges F (1997) Allergic cross-reactions between cat and pig serum albumin. Study at the protein and DNA levels. *Allergy* **52**: 179-187

Hill AB (1965) The environment and disease: association or causation? Proceedings of the Royal Society of Medicine: Section of Occupational Health.

Hill JR, Burghardt RC, Jones K, Long CR, Looney CR, Shin T, Spencer TE, Thompson JA, Winger QA, Westhusin ME (2000) Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. *Biol Reprod* **63**: 1787-1794

Hill JR, Edwards JF, Sawyer N, Blackwell C, Cibelli JB (2001) Placental anomalies in a viable cloned calf. *Cloning* **3**: 83-88

Hill JR, Roussel AJ, Cibelli JB, Edwards JF, Hooper NL, Miller MW, Thompson JA, Looney CR, Westhusin ME, Robl JM, Stice SL (1999) Clinical and pathologic features of cloned transgenic calves and fetuses (13 case studies). *Theriogenology* **51**: 1451-1465

Hill JR, Schlafer DH, Fisher PJ, Davies CJ (2002) Abnormal expression of trophoblast major histocompatibility complex class I antigens in cloned bovine pregnancies is associated with a pronounced endometrial lymphocytic response. *Biol Reprod* **67**: 55-63

Hill JR, Winger QA, Burghardt RC, Westhusin ME (2001) Bovine nuclear transfer embryo development using cells derived from a cloned fetus. *Anim Reprod Sci* **67**: 17-26

Hill JR, Winger QA, Long CR, Looney CR, Thompson JA, Westhusin ME (2000) Development rates of male bovine nuclear transfer embryos derived from adult and fetal cells. *Biol Reprod* **62**: 1135-1140

Hinrichs K (2005) Update on equine ICSI and cloning. *Theriogenology* **64**: 535-541

Hinrichs K, Choi YH, Love CC, Chung YG, Varner DD (2006) Production of horse foals via direct injection of roscovitine-treated donor cells and activation by injection of sperm extract. *Reproduction* **131**: 1063-1072

Hochedlinger K, Jaenisch R (2002) Monoclonal mice generated by nuclear transfer from mature B and T donor cells. *Nature* **415**: 1035-1038

Hoelker M, Schmoll F, Schneider H, Rings F, Gilles M, Tesfaye D, Jennen D, Tholen E, Griese J, Schellander K (2006) Bovine blastocyst diameter as a morphological tool to predict embryo cell counts, embryo sex, hatching ability and developmental characteristics after transfer to recipients. *Reprod Fertil Dev* **18**: 551-557

Hoffert KA, Batchelder CA, Bertolini M, Moyer AL, Famula TR, Anderson DL, Anderson GB (2005) Measures of maternal-fetal interaction in day-30 bovine pregnancies derived from nuclear transfer. *Cloning Stem Cells* **7**: 289-305

Hohlweg U, Doerfler W (2001) On the fate of plant or other foreign genes upon the uptake in food or after intramuscular injection in mice. *Mol Genet Genomics* **265**: 225-233

Holliday R (2005) DNA methylation and epigenotypes. *Biochemistry (Mosc)* **70**: 500-504

Hornen N, Kues WA, Carnwath JW, Lucas-Hahn A, Petersen B, Hassel P, Niemann H (2007) Production of viable pigs from fetal somatic stem cells. *Cloning Stem Cells* **9**: 364-373

Hou YP, Dai YP, Zhu SE, Zhu HB, Wu TY, Gong GC, Wang HP, Wang LL, Liu Y, Li R, Wan R, Li N (2005) Bovine oocytes vitrified by the open pulled straw method and used for somatic cell cloning supported development to term. *Theriogenology* **64**: 1381-1391

Hua S, Zhang Z, Zhang C, Zhang Y (2007) An improved enucleation method of bovine somatic cell nuclear transfer. *J Genet Genomics* **34**: 491-496

Humpherys D, Eggan K, Akutsu H, Friedman A, Hochedlinger K, Yanagimachi R, Lander ES, Golub TR, Jaenisch R (2002) Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei. *Proc Natl Acad Sci U S A* **99**: 12889-12894

Humpherys D, Eggan K, Akutsu H, Hochedlinger K, Rideout WM, III, Biniszkiewicz D, Yanagimachi R, Jaenisch R (2001) Epigenetic instability in ES cells and cloned mice. *Science* **293** : 95-97

Ideta A, Urakawa M, Aoyagi Y, Saeki K (2005) Early morphological nuclear events and developmental capacity of embryos reconstructed with fetal fibroblasts at the M or G1 phase after intracytoplasmic nuclear injection in cattle. *J Reprod Dev* **51**: 187-194

IFIC Survey: Food Biotechnology Not A Top-Of-Mind Concern for American Consumers. 2005. International Food Information Council.

Iguma LT, Lisauskas SF, Melo EO, Franco MM, Pivato I, Vianna GR, Sousa RV, Dode MA, Aragao FJ, Rech EL, Rumpf R (2005) Development of bovine embryos reconstructed by nuclear transfer of transfected and non-transfected adult fibroblast cells. *Genet Mol Res* **4**: 55-66

Ikumi S, Asada M, Sawai K, Fukui Y (2003) Effect of activation methods for bovine oocytes after intracytoplasmic injection. *J Reprod Dev* **49**: 37-43

Illmensee K, Levanduski M, Zavos PM (2006) Evaluation of the embryonic preimplantation potential of human adult somatic cells via an embryo interspecies bioassay using bovine oocytes. *Fertil Steril* **85 Suppl 1**: 1248-1260

Im GS, Seo JS, Hwang IS, Kim DH, Kim SW, Yang BC, Yang BS, Lai L, Prather RS (2006) Development and apoptosis of pre-implantation porcine nuclear transfer embryos activated with different combination of chemicals. *Mol Reprod Dev* **73**: 1094-1101

Inoue F, Matsuda J, Ohkoshi K, Furusawa T, Takahashi S, Sasada H, Sato E, Tokunaga T (2006) Differences in gene expression patterns between somatic cell nuclear transfer embryos constructed with either rabbit granulosa cells or their derivatives. *Anim Reprod Sci* **93**: 76-87

Inoue K, Kohda T, Lee J, Ogonuki N, Mochida K, Noguchi Y, Tanemura K, Kaneko-Ishino T, Ishino F, Ogura A (2002) Faithful expression of imprinted genes in cloned mice. *Science* **295** : 297

Inoue K, Ogonuki N, Mochida K, Yamamoto Y, Takano K, Kohda T, Ishino F, Ogura A (2003) Effects of donor cell type and genotype on the efficiency of mouse somatic cell cloning. *Biol Reprod* **69**: 1394-1400

Jaenisch R (2004) Human cloning - the science and ethics of nuclear transplantation. *N Engl J Med* **351**: 2787-2791

Jaenisch R, Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* **33 Suppl**: 245-254

Jaenisch R, Eggan K, Humpherys D, Rideout W, Hochedlinger K (2002) Nuclear cloning, stem cells, and genomic reprogramming. *Cloning Stem Cells* **4**: 389-396

Jaenisch R, Hochedlinger K, Blelloch R, Yamada Y, Baldwin K, Eggan K (2004) Nuclear cloning, epigenetic reprogramming, and cellular differentiation. *Cold Spring Harb Symp Quant Biol* **69**: 19-27

Jakobsen AS, Thomsen PD, Avery B (2006) Few polyploid blastomeres in morphologically superior bovine embryos produced in vitro. *Theriogenology* **65**: 870-881

Jang G, Bhuiyan MM, Jeon HY, Ko KH, Park HJ, Kim MK, Kim JJ, Kang SK, Lee BC, Hwang WS (2006) An approach for producing transgenic cloned cows by nuclear transfer of cells transfected with human alpha 1-antitrypsin gene. *Theriogenology* **65**: 1800-1812

Jang G, Jeon HY, Ko KH, Park HJ, Kang SK, Lee BC, Hwang WS (2005) Developmental competence and gene expression in preimplantation bovine embryos derived from somatic cell nuclear transfer using different donor cells. *Zygote* **13**: 187-195

Jang G, Kim MK, Oh HJ, Hossein MS, Fibrianto YH, Hong SG, Park JE, Kim JJ, Kim HJ, Kang SK, Kim DY, Lee BC (2007) Birth of viable female dogs produced by somatic cell nuclear transfer. *Theriogenology* **67**: 941-947

Japan Livestock Technology Association. Project report of an investigation on the properties of products from cloned cattle: An urgent study project for the utlization of cloned cattle. 9-10-2002. Japan Livestock Technology Association.
Ref Type: Report

Jenness R (1988) Composition of Milk. In *Fundamentals of Dairy Chemistry*, Wong NP, Jenness R, Keeney M, Marth EH (eds) pp 1-38. Van Nostrand Reinhold Company: New York

Jeon HY, Hyun SH, Lee GS, Kim HS, Kim S, Jeong YW, Kang SK, Lee BC, Han JY, Ahn C, Hwang WS (2005) The analysis of telomere length and telomerase activity in cloned pigs and cows. *Mol Reprod Dev* **71**: 315-320

Jeong YW, Park SW, Hossein MS, Kim S, Kim JH, Lee SH, Kang SK, Lee BC, Hwang WS (2006) Antiapoptotic and embryotrophic effects of [alpha]-tocopherol and l-ascorbic acid on

porcine embryos derived from in vitro fertilization and somatic cell nuclear transfer. *Theriogenology* **66**: 2104-2112

Jiang L, Carter DB, Xu J, Yang X, Prather RS, Tian XC (2004) Telomere lengths in cloned transgenic pigs. *Biol Reprod* **70**: 1589-1593

Jiang L, Jobst P, Lai L, Samuel M, Ayares D, Prather RS, Tian XC (2007) Expression levels of growth-regulating imprinted genes in cloned piglets. *Cloning Stem Cells* **9**: 97-106

Jiang Y, Chen T, Nan CL, Ouyang YC, Sun QY, Chen DY (2005) In vitro culture and mtDNA fate of ibex-rabbit nuclear transfer embryos. *Zygote* **13**: 233-240

Jiang Y, Chen T, Wang K, Jiang MX, Liu SZ, Ouyang YC, Sun QY, Chen DY (2006) Different fates of donor mitochondrial DNA in bovine-rabbit and cloned bovine-rabbit reconstructed embryos during preimplantation development. *Front Biosci* **11**: 1425-1432

Jian-Quan C, Juan C, Xu-Jun X, Guo-Hui L, Si-Guo L, Hong-Ying S, You-Bing W, Guo-Xiang C (2007) Effect of cytoplast on the development of inter-subspecies nuclear transfer reconstructed goat embryo. *Mol Reprod Dev* **74**: 568-573

Jiao F, Yan JB, Yang XY, Li H, Wang Q, Huang SZ, Zeng F, Zeng YT (2007) Effect of oocyte mitochondrial DNA haplotype on bovine somatic cell nuclear transfer efficiency. *Mol Reprod Dev*

Jones PA, Takai D (2001) The role of DNA methylation in mammalian epigenetics. *Science* **293**: 1068-1070

Jouneau A, Renard JP (2003) Reprogramming in nuclear transfer. *Curr Opin Genet Dev* **13**: 486-491

Kang YK, Koo DB, Park JS, Choi YH, Chung AS, Lee KK, Han YM (2001) Aberrant methylation of donor genome in cloned bovine embryos. *Nat Genet* **28**: 173-177

Kang YK, Koo DB, Park JS, Choi YH, Kim HN, Chang WK, Lee KK, Han YM (2001) Typical demethylation events in cloned pig embryos. Clues on species-specific differences in epigenetic reprogramming of a cloned donor genome. *J Biol Chem* **276**: 39980-39984

Kang YK, Koo DB, Park JS, Choi YH, Lee KK, Han YM (2001) Influence of oocyte nuclei on demethylation of donor genome in cloned bovine embryos. *FEBS Lett* **499**: 55-58

Kang YK, Lee KK, Han YM (2003) Reprogramming DNA methylation in the preimplantation stage: peeping with Dolly's eyes. *Curr Opin Cell Biol* **15**: 290-295

Kanka J (2003) Gene expression and chromatin structure in the pre-implantation embryo. *Theriogenology* **59**: 3-19

Kasai K, Sano F, Miyashita N, Watanabe S, Nagai T (2007) Comparison of the growth performances of offspring produced by a pair of cloned cattle and their nuclear donor animals. *J Reprod Dev* **53**: 135-142

Katayama M, Zhong Z, Lai L, Sutovsky P, Prather RS, Schatten H (2006) Mitochondrial distribution and microtubule organization in fertilized and cloned porcine embryos: implications for developmental potential. *Dev Biol* **299**: 206-220

Kato Y, Tani T, Sotomaru Y, Kurokawa K, Kato J, Doguchi H, Yasue H, Tsunoda Y (1998) Eight calves cloned from somatic cells of a single adult. *Science* **282**: 2095-2098

Kato Y, Tani T, Tsunoda Y (2000) Cloning of calves from various somatic cell types of male and female adult, newborn and fetal cows. *J Reprod Fertil* **120**: 231-237

Katska L, Bochenek M, Kania G, Rynska B, Smorag Z (2002) Flow cytometric cell cycle analysis of somatic cells primary cultures established for bovine cloning. *Theriogenology* **58**: 1733-1744

Kaufmann W, Hagemeister H (1987) Composition of milk. In *Dairy Cattle Production*, Gravert HO (ed) Elsevier Science Publishers: Amsterdam

Kazmer GW, Zinn SA, Rycroft H, Campbell RM (1992) Serum growth hormone in and semen characteristics of proven AI dairy sires after administration of growth hormone-releasing factor. *Can J Anim Sci* **72**: 963

Keefer CL, Baldassarre H, Keyston R, Wang B, Bhatia B, Bilodeau AS, Zhou JF, Leduc M, Downey BR, Lazaris A, Karatzas CN (2001) Generation of dwarf goat (Capra hircus) clones following nuclear transfer with transfected and nontransfected fetal fibroblasts and in vitro-matured oocytes. *Biol Reprod* **64**: 849-856

Keefer CL, Bhatia B, Kafidi N (2001) Abstract for poster presentation : Student competition finalists. *Theriogenology* **55**: 274

Keefer CL, Keyston R, Lazaris A, Bhatia B, Begin I, Bilodeau AS, Zhou FJ, Kafidi N, Wang B, Baldassarre H, Karatzas CN (2002) Production of cloned goats after nuclear transfer using adult somatic cells. *Biol Reprod* **66**: 199-203

Keefer CL, Stice SL, Matthews DL (1994) Bovine inner cell mass cells as donor nuclei in the production of nuclear transfer embryos and calves. *Biol Reprod* **50**: 935-939

Kelly TL, Trasler JM (2004) Reproductive epigenetics. *Clin Genet* **65**: 247-260

Kessler DA, Taylor MR, Maryanski JH, Flamm EL, Kahl LS (1992) The safety of foods developed by biotechnology. *Science* 1747-1749

Kikyo N, Wolffe AP (2000) Reprogramming nuclei: insights from cloning, nuclear transfer and heterokaryons. *J Cell Sci* **113 (Pt 1)**: 11-20

Kim HR, Kang JK, Yoon JT, Seong HH, Jung JK, Lee HM, Sik PC, Jin DI (2005) Protein profiles of bovine placenta derived from somatic cell nuclear transfer. *Proteomics* **5**: 4264-4273

Kim HS, Lee GS, Kim JH, Kang SK, Lee BC, Hwang WS (2006) Expression of leptin ligand and receptor and effect of exogenous leptin supplement on in vitro development of porcine embryos. *Theriogenology* **65**: 831-844

Kim MK, Jang G, Oh HJ, Yuda F, Kim HJ, Hwang WS, Hossein MS, Kim JJ, Shin NS, Kang SK, Lee BC (2007) Endangered wolves cloned from adult somatic cells. *Cloning Stem Cells* **9**: 130-137

Kim S, Lee SH, Kim JH, Jeong YW, Hashem MA, Koo OJ, Park SM, Lee EG, Hossein MS, Kang SK, Lee BC, Hwang WS (2006) Anti-apoptotic effect of insulin-like growth factor (IGF)-I and its receptor in porcine preimplantation embryos derived from in vitro fertilization and somatic cell nuclear transfer. *Mol Reprod Dev* **73**: 1523-1530

King KK, Seidel GE, Jr., Elsden RP (1985) Bovine embryo transfer pregnancies. I. Abortion rates and characteristics of calves. *J Anim Sci* **61**: 747-757

King T, De Sousa PA (2006) Maintenance of pregnancy in pigs with limited viable embryos. *Methods Mol Biol* **348**: 79-90

King TJ, Dobrinsky JR, Zhu J, Finlayson HA, Bosma W, Harkness L, Ritchie WA, Travers A, McCorquodale C, Day BN, Dinnyes A, De Sousa PA, Wilmut I (2002) Embryo development and establishment of pregnancy after embryo transfer in pigs: coping with limitations in the availability of viable embryos. *Reproduction* **123**: 507-515

King WA, Coppola G, Alexander B, Mastromonaco G, Perrault S, Nino-Soto MI, Pinton A, Joudrey EM, Betts DH (2006) The impact of chromosomal alteration on embryo development. *Theriogenology* **65**: 166-177

Kishi M, Itagaki Y, Takakura R, Imamura M, Sudo T, Yoshinari M, Tanimoto M, Yasue H, Kashima N (2000) Nuclear transfer in cattle using colostrum-derived mammary gland epithelial cells and ear-derived fibroblast cells. *Theriogenology* **54**: 675-684

Kishi M, Takakura R, Nagao Y, Saeki K, Takahashi Y (2007) Effect of embryonic cell cycle of nuclear donor embryos on the efficiency of nuclear transfer in Japanese black cattle. *Zygote* **15**: 165-171

Kishigami S, Mizutani E, Ohta H, Hikichi T, Thuan NV, Wakayama S, Bui HT, Wakayama T (2006) Significant improvement of mouse cloning technique by treatment with trichostatin A after somatic nuclear transfer. *Biochem Biophys Res Commun* **340**: 183-189

Kitiyanant Y, Saikhun J, Chaisalee B, White KL, Pavasuthipaisit K (2001) Somatic cell cloning in Buffalo (Bubalus bubalis): effects of interspecies cytoplasmic recipients and activation procedures. *Cloning Stem Cells* **3**: 97-104

Klein C, Bauersachs S, Ulbrich SE, Einspanier R, Meyer HH, Schmidt SE, Reichenbach HD, Vermehren M, Sinowatz F, Blum H, Wolf E (2006) Monozygotic twin model reveals novel embryo-induced transcriptome changes of bovine endometrium in the preattachment period. *Biol Reprod* **74**: 253-264

Knight TW, Lambert MG, Devantier BP, Betteridge K (2001) Calf survival from embryo transfer-induced twinning in dairy-beef cows and the effects of synchronised calving. *Anim Reprod Sci* **68**: 1-12

Knobil E, Neill JD (1998) *Encyclopedia of Reproduction*. Academic Press:

Koketsu Y (2007) Longevity and efficiency associated with age structures of female pigs and herd management in commercial breeding herds. *J Anim Sci* **85**: 1086-1091

Kranendonk G, Van der MH, Fillerup M, Hopster H (2007) Social rank of pregnant sows affects their body weight gain and behavior and performance of the offspring. *J Anim Sci* **85**: 420-429

Kremenskoy M, Kremenska Y, Suzuki M, Imai K, Takahashi S, Hashizume K, Yagi S, Shiota K (2006) Epigenetic characterization of the CpG islands of bovine Leptin and POU5F1 genes in cloned bovine fetuses. *J Reprod Dev* **52**: 277-285

Kremenskoy M, Kremenska Y, Suzuki M, Imai K, Takahashi S, Hashizume K, Yagi S, Shiota K (2006) DNA methylation profiles of donor nuclei cells and tissues of cloned bovine fetuses. *J Reprod Dev* **52**: 259-266

Kruip T, den Daas JHG (1997) In vitro produced and cloned embryos: Effects on pregnancy, parturition and offspring. *Theriogenology* **47**: 43-52

Kruip TA, Bevers MM, Kemp B (2000) Environment of oocyte and embryo determines health of IVP offspring. *Theriogenology* **53**: 611-618

Kubota C, Tian XC, Yang X (2004) Serial bull cloning by somatic cell nuclear transfer. *Nat Biotechnol* **22**: 693-694

Kubota C, Yamakuchi H, Todoroki J, Mizoshita K, Tabara N, Barber M, Yang X (2000) Six cloned calves produced from adult fibroblast cells after long-term culture. *Proc Natl Acad Sci U S A* **97**: 990-995

Kuhholzer-Cabot B, Brem G (2002) Aging of animals produced by somatic cell nuclear transfer. *Exp Gerontol* **37**: 1317-1323

Kuhn MT, Hutchison JL, Wiggans GR (2006) Characterization of Holstein heifer fertility in the United States. *J Dairy Sci* **89**: 4907-4920

Kummerfeld HL, Oltenacu EA, Foote RH (1978) Embryonic mortality in dairy cows estimated by nonreturns to service, estrus, and cyclic milk progesterone patterns. *J Dairy Sci* **61**: 1773-1777

Kun Z, Shaohua W, Yufang M, Yankun L, Hengxi W, Xiuzhu S, Yonghui Z, Yan L, Yunping D, Lei Z, Ning L (2006) Effects of leptin supplementation in in vitro maturation medium on meiotic maturation of oocytes and preimplantation development of parthenogenetic and cloned embryos in pigs. *Anim Reprod Sci*

Kuran M, McEvoy TG, Young LE, Broadbent PJ, Robinson JJ, Sinclair KD (2000) Ovine fetal development follwoing different periods of in vitro culture. *Theriogenology* **53**: 275

Lacham-Kaplan O, Diamente M, Pushett D, Lewis I, Trounson A (2000) Developmental competence of nuclear transfer cow oocytes after direct injection of fetal fibroblast nuclei. *Cloning* **2**: 55-62

Lagutina I, Lazzari G, Duchi R, Colleoni S, Ponderato N, Turini P, Crotti G, Galli C (2005) Somatic cell nuclear transfer in horses: effect of oocyte morphology, embryo reconstruction method and donor cell type. *Reproduction* **130**: 559-567

Lagutina I, Lazzari G, Duchi R, Turini P, Tessaro I, Brunetti D, Colleoni S, Crotti G, Galli C (2007) Comparative aspects of somatic cell nuclear transfer with conventional and zona-free method in cattle, horse, pig and sheep. *Theriogenology* **67**: 90-98

Lagutina I, Lazzari G, Galli C (2006) Birth of cloned pigs from zona-free nuclear transfer blastocysts developed in vitro before transfer. *Cloning Stem Cells* **8**: 283-293

Lai L, Kolber-Simonds D, Park KW, Cheong HT, Greenstein JL, Im GS, Samuel M, Bonk A, Rieke A, Day BN, Murphy CN, Carter DB, Hawley RJ, Prather RS (2002) Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. *Science* **295**: 1089-1092

Laible G, Brophy B, Knighton D, Wells DN (2007) Compositional analysis of dairy products derived from clones and cloned transgenic cattle. *Theriogenology* **67**: 166-177

Lan GC, Chang ZL, Luo MJ, Jiang YL, Han D, Wu YG, Han ZB, Ma SF, Tan JH (2006) Production of cloned goats by nuclear transfer of cumulus cells and long-term cultured fetal fibroblast cells into abattoir-derived oocytes. *Mol Reprod Dev* **73**: 834-840

Landmann JK, Jillella D, O'Donoghue PJ, McGowan MR (2002) Confirmation of the prevention of vertical transmission of Neospora caninum in cattle by the use of embryo transfer. *Aust Vet J* **80**: 502-503

Landry AM, Landry DJ, Gentry LR, Green HL, Reggio B, Koonce KL, Echelard Y, Godke RA (2005) Endocrine profiles and growth patterns of cloned goats. *Cloning Stem Cells* **7**: 214-225

Lane N, Dean W, Erhardt S, Hajkova P, Surani A, Walter J, Reik W (2003) Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. *Genesis* **35**: 88-93

Lanza RP, Cibelli JB, Blackwell C, Cristofalo VJ, Francis MK, Baerlocher GM, Mak J, Schertzer M, Chavez EA, Sawyer N, Lansdorp PM, West MD (2000) Extension of cell life-span and telomere length in animals cloned from senescent somatic cells. *Science* **288**: 665-669

Lanza RP, Cibelli JB, Faber D, Sweeney RW, Henderson B, Nevala W, West MD, Wettstein PJ (2001) Cloned cattle can be healthy and normal. *Science* **294**: 1893-1894

Latham KE, Gao S, Han Z (2007) Somatic cell nuclei in cloning: strangers traveling in a foreign land. *Adv Exp Med Biol* **591**: 14-29

Laurincik J, Maddox-Hyttel P (2007) Nucleolar remodeling in nuclear transfer embryos. *Adv Exp Med Biol* **591**: 84-92

Lawrence JL, Schrick FN, Hopkins FM, Welborn MG, McCracken MD, Sonstegard T, Wilson TJ, Edwards JL (2005) Fetal losses and pathologic findings of clones derived from serum-starved versus serum-fed bovine ovarian granulosa cells. *Reprod Biol* **5**: 171-184

Lay DC, Jr., Haussmann MF, Buchanan HS, Daniels MJ (1999) Danger to pigs due to crushing can be reduced by the use of a simulated udder. *J Anim Sci* **77**: 2060-2064

Lazzari G, Colleoni S, Giannelli SG, Brunetti D, Colombo E, Lagutina I, Galli C, Broccoli V (2006) Direct derivation of neural rosettes from cloned bovine blastocysts: a model of early neurulation events and neural crest specification in vitro. *Stem Cells* **24** : 2514-2521

Lazzari G, Wrenzycki C, Herrmann D, Duchi R, Kruip T, Niemann H, Galli C (2002) Cellular and molecular deviations in bovine in vitro-produced embryos are related to the large offspring syndrome. *Biol Reprod* **67**: 767-775

Le Bourhis D, Chesne P, Nibart M, Marchal J, Humblot P, Renard JP, Heyman Y (1998) Nuclear transfer from sexed parent embryos in cattle: efficiency and birth of offspring. *J Reprod Fertil* **113**: 343-348

Le Roith D, Scavo L, Butler A (2001) What is the role of circulating IGF-I? *Trends Endocrinol Metab* **12**: 48-52

Lee BC, Kim MK, Jang G, Oh HJ, Yuda F, Kim HJ, Hossein MS, Kim JJ, Kang SK, Schatten G, Hwang WS (2005) Dogs cloned from adult somatic cells. *Nature* **436**: 641

Lee E, Lee SH, Kim S, Jeong YW, Kim JH, Koo OJ, Park SM, Hashem MA, Hossein MS, Son HY, Lee CK, Hwang WS, Kang SK, Lee BC (2006) Analysis of nuclear reprogramming in cloned miniature pig embryos by expression of Oct-4 and Oct-4 related genes. *Biochem Biophys Res Commun* **348**: 1419-1428

Lee GS, Hyun SH, Kim HS, Kim DY, Lee SH, Lim JM, Lee ES, Kang SK, Lee BC, Hwang WS (2003) Improvement of a porcine somatic cell nuclear transfer technique by optimizing donor cell and recipient oocyte preparations. *Theriogenology* **59**: 1949-1957

Lee GS, Kim HS, Hyun SH, Jeon HY, Nam DH, Jeong YW, Kim S, Kim JH, Kang SK, Lee BC, Hwang WS (2005) Effect of epidermal growth factor in preimplantation development of porcine cloned embryos. *Mol Reprod Dev* **71**: 45-51

Lee GS, Kim HS, Lee SH, Kim DY, Seo KM, Hyun SH, Kang SK, Lee BC, Hwang WS (2005) Successful surgical correction of anal atresia in a transgenic cloned piglet. *J Vet Sci* **6**: 243-245

Lee JH, Campbell KH (2006) Effects of enucleation and caffeine on maturation-promoting factor (MPF) and mitogen-activated protein kinase (MAPK) activities in ovine oocytes used as recipient cytoplasts for nuclear transfer. *Biol Reprod* **74**: 691-698

Lee KB, Niwa K (2006) Fertilization and development in vitro of bovine oocytes following intracytoplasmic injection of heat-dried sperm heads. *Biol Reprod* **74**: 146-152

Lee RS, Peterson AJ, Donnison MJ, Ravelich S, Ledgard AM, Li N, Oliver JE, Miller AL, Tucker FC, Breier B, Wells DN (2004) Cloned cattle fetuses with the same nuclear genetics are more variable than contemporary half-siblings resulting from artificial insemination and exhibit fetal and placental growth deregulation even in the first trimester. *Biol Reprod* **70**: 1-11

Lee SL, Kumar BM, Kim JG, Ock SA, Jeon BG, Balasubramanian S, Choe SY, Rho GJ (2007) Cellular composition and viability of cloned bovine embryos using exogene-transfected somatic cells. *Reprod Domest Anim* **42**: 44-52

Lee SL, Ock SA, Yoo JG, Kumar BM, Choe SY, Rho GJ (2005) Efficiency of gene transfection into donor cells for nuclear transfer of bovine embryos. *Mol Reprod Dev* **72**: 191-200

Lee SY, Park JY, Choi YJ, Cho SK, Ahn JD, Kwon DN, Hwang KC, Kang SJ, Paik SS, Seo HG, Lee HT, Kim JH (2007) Comparative proteomic analysis associated with term placental insufficiency in cloned pig. *Proteomics*

Levick SE (2007) From Xenopus to Oedipus: "Dolly," human cloning, and psychological and social "clone-ness". *Cloning Stem Cells* **9**: 33-39

Lewis IM, Peura TT, Trounson AO (1998) Large-scale applications of cloning technologies for agriculture: an industry perspective. *Reprod Fertil Dev* **10**: 677-681

Li E (2002) Chromatin modification and epigenetic reprogramming in mammalian development. *Nat Rev Genet* **3**: 662-673

Li GP, Bunch TD, White KL, Rickords L, Liu Y, Sessions BR (2006) Denuding and centrifugation of maturing bovine oocytes alters oocyte spindle integrity and the ability of cytoplasm to support parthenogenetic and nuclear transfer embryo development. *Mol Reprod Dev* **73**: 446-451

Li GP, Liu Y, White KL, Bunch TD (2005) Cytogenetic analysis of diploidy in cloned bovine embryos using an improved air-dry karyotyping method. *Theriogenology* **63**: 2434-2444

Li GP, White KL, Aston KI, Meerdo LN, Bunch TD (2004) Conditioned medium increases the polyploid cell composition of bovine somatic cell nuclear-transferred blastocysts. *Reproduction* **127**: 221-228

Li N, Wells DN, Peterson AJ, Lee RS (2005) Perturbations in the biochemical composition of fetal fluids are apparent in surviving bovine somatic cell nuclear transfer pregnancies in the first half of gestation. *Biol Reprod* **73**: 139-148

Li R, Lai L, Wax D, Hao Y, Murphy CN, Rieke A, Samuel M, Linville ML, Korte SW, Evans RW, Turk JR, Kang JX, Witt WT, Dai Y, Prather RS (2006) Cloned transgenic swine via in vitro production and cryopreservation. *Biol Reprod* **75**: 226-230

Li S, Chen X, Fang Z, Shi J, Sheng HZ (2006) Rabbits generated from fibroblasts through nuclear transfer. *Reproduction* **131**: 1085-1090