Appendix G: Unpublished Data

In July 2001, the Center issued a CVM Update requesting that clone producers not introduce meat or milk from clones or their progeny into food or feed until the Agency had time to complete a review of the safety of those food products. At that time, CVM also requested that clone producers submit data to the Center on the health of clones and their progeny, and on meat and milk composition of clones and progeny. Two large datasets were submitted by two companies: Cyagra, Inc. and ViaGen, Inc. Reviews of these data are provided in Appendices E and F, respectively. In addition, several small datasets were submitted to the Center, and included tables and reports of veterinarians and technicians for neonatal cattle and swine, as well as data on juvenile development, reproductive function and maturity in cattle. These data were originally included with Chapter V in the release of the Draft Risk Assessment on December 28, 2006, and are now presented in their entirety in this Appendix and reviewed below. One additional dataset has also been submitted by J.L. Edwards from the University of Tennessee, who also provided additional information in a teleconference on September 12, 2007.

A. Perinatal Development in Cattle Clones (Node 2)

Body temperature, pulse and respiration rate data were submitted covering the first 72 hours of life for 19 clone calves of unknown breed(s) from a commercial cloning company (Table G-1).

Mean ± SD for body temperature, pulse and respiration at birth for the 19 calves were 103.0 ± 1.2 ˚ F, 95.2 ± 30.34 beats/min, and 53.9 ± 19.4 breaths/min, respectively. At 24 hours, means were 101.8 ± 0.7 ˚ F, 133.1 ± 19.2 beats/min, and 57.4 ± 21.1 breaths/min. At 48 hours, means were 102.0 ± 0.5 ˚ F, 135.8 ± 17.9 beats/min, and 56.1 ± 24.8 breaths/min. Values for one calf were not available for the 72 hour measurements, such that means and standard deviations represent 18 calves. Those values were 102.7 ± 0.7 ˚ F, 138.6 ± 19.3 beats/min, and 53.1 ± 20.9 breaths/min. Heart and respiration rates vary with age. Respiration rates in growing steers (age not specified) were noted to be 79 ± 3.2 breaths/min in one study (Nihsen et al. 2004). Breukelman et al. (2004) noted basal fetal heart rates in late gestation AI pregnancies to be 111.6 ± 1.4 beats/min. By comparison, basal heart rate of three week old heifer calves averaged 88.1 ± 4.04 beats/min (Van Reenen et al. 2005).

Table G-1: Pulse, Respiration, and Body Temperature of Nineteen Cattle Clones of Unknown Breeds During the First 72 After Birth

Calf ID	Calving Date	Temperature (F)				Pulse				Respiration			
		Birth	24h	48h	72h	Birth	24h	48h	72h	Birth	24h	48h	72h
1	2/12/2001	104.8	103	102.2	102.6	54	132	144	116	48	44	48	84
2	3/28/2001	101.2	101.7	101.7	101.8	120	114	138	138	36	42	42	36
3	4/10/2001	103	101.6	101.4	103.6	100	120	126	140	46	44	44	48
4	4/12/2001	104.3	101.2	101.6	102.6	64	120	132	140	30	48	44	36
5	4/13/2001	102.6	101.1	102	103.6	100	120	120	140	44	56	48	48
6	4/10/2001	102.9	101.5	102.3	102.5	116	144	128	126	68	54	56	39
7	4/11/2001	100.5	101.1	101.7	102.9	112	120	160	152	54	48	40	40
8	1/15/2002	102.4	102	101.8	102	60	144	140	128	60	48	28	20
9	1/30/2002	103.2	101.3	102	101.1	128	136	140	132	98	56	52	44
10	1/31/2002	105.2	101.5	101.6	102.2	150	140	140	115	36	32	24	40
11	1/29/2002	102.4	102	102.5	102.6	66	132	126	138	66	108	102	78
12	3/27/2002	102.5	102.5	102	102.4	60	192	104	156	60	54	40	56
13	3/21/2002	103.4	100.8	101.3	103	108	108	132	156	72	36	30	24
14	4/9/2002	103.9	101.3	102.1	102.9	40	114	120	120	24	80	84	72
15	4/4/2002	104	102.2	102.2		90	132	180		24	56	72	
16	5/1/2002	103.4	101.3	102.4	103	90	120	120	102	50	48	68	68
17	4/30/2002	101.3	103.2	101.5	102	120	140	120	180	78	88	64	66
18	11/11/2002	103.3	103.1	103.2	103.4	100	150	150	160	70	100	120	60
19	11/12/2002	102	101.6	101.8	103.8	130	150	160	156	60	48	60	96

The birth records of two Holstein heifer clones were submitted by a private cloning firm. Both heifers were delivered by C-section. One calf was a breech position (posterior presentation with hind limbs under body); the other calf was in a normal posterior position, with hind limbs extended. The placentae of these calves were described as normal, with some large and some small placentomes described for one placenta. Calves were described as normal, weighing 45 and 47.7 kg each at time of delivery. Both calves had normal umbilici. Some fluid was noted in the lungs of both calves, but they were described as breathing normally, and although some meconium staining was noted, there was no indication that meconium had been inhaled. Body temperatures were 100 and 102.6˚F, slightly below and above the average temperature for cattle. Blood glucose, packed cell volume (PCV), blood total protein, and IgG concentrations were monitored for the first 23 to 27 hours after birth (Table G-2). Blood glucose was low for both calves prior to first feeding, then increased to normal levels by the second feeding. Total protein also increased steadily following feeding, and IgG levels were listed as "> 10" after the first colostrum feeding. The units for IgG measurements were not provided, and PCV values were the

only hematology data provided, so these data are difficult to interpret. Total protein and serum glucose values are comparable to age-matched non-clone cattle in the Cyagra dataset by the second post-feeding blood sample.

Table G-2: Reported blood values for Two Holstein Heifer Clones

	Calf 1			Calf 2		
	Pre-feeding	**1st feeding**	**2nd feeding**	**Pre-feeding**	**1st feeding**	**2nd feeding**
Glucose	20	35	93	21	29	83
PCV	29	28	25	30	33	29
Total Protein	5	5.3	7.5	4.8	5.0	6.0
IgG	N/A	>10	>10	N/A	>10	>10

B. Juvenile Development in Cattle Clones (Node 3)

A private veterinary clinic submitted hematology and clinical chemistry data on three bull clones, ranging in age from 5 to 7 months at the time of the first sampling. Animals were sampled a total of three times within a six week period. Most variables measured were within the reference range used by the diagnostic lab conducting the tests (Marshfield Laboratories, Marshfield, WI). Values outside the reference range for the testing laboratory are listed in Tables G-3 a, b, and c.

CVM contacted Marshfield Laboratories on September 21, 2005, regarding the source of their reference range. According to the laboratory, the reference range for hematology and clinical chemistry was established on blood samples taken from female dairy animals between 1 and 8 years of age. As discussed in Appendix E, it is important that the selected reference range is appropriate to the animals being tested. In this case, the use of a reference range established using post-pubertal, near-adult and adult females may not provide an appropriate comparison for pre-pubertal, rapidly growing males. As no contemporary comparator animals were sampled, other published reference ranges (Meyer and Harvey 2004; Green 1998; Duncan and Prasse 2003) were used for cross-comparison. Results indicated that one clone (Clone #3) on the second sampling date had one analyte that was outside any of the reference ranges used. For Clone #3, cholesterol was low on October 13. However, all of this animal's hematology and clinical chemistry values were considered within published ranges on the third sampling date, approximately two weeks later. Because serum cholesterol can be affected by diet and time since the last meal, the single low value for this animal was judged not to be biologically relevant.

Table G-3a: Hematology and Clinical Chemistry for Three Holstein Bulls (Bull #1)

								RMIN	RMAX	Units
Date Section 1 Collected			**9/22/03**		**10/13/03**		**10/27/03**			
Hemogram-Vet (VCLT)										
	Red Blood Cell Count	.	7.03					5	10	x 10^6/uL
	Hemoglobin	.	9.10					8	15	g/dL
	Hematocrit	.	29.00					24	46	%
	Mean Corpuscular Volume	.	41.20					40	60	fL
	Mean Corpuscular Hemoglobin	.	12.90					11	17	pg
	Mean Corpuscular Hgb Conc.	.	31.40					30	36	g/dL
	Red Cell Distribution Width	L	21.60					26	30	%
	Platelet Count	H	720.00					230	690	x 10^3/uL
	White Blood Cell Count	.	5.30					4	12	x 10^3/uL
	Seg. Neutrophil Absolute #	.	1.64					0.6	4	x 10^3/uL
	Banded Neutrophil Absolute #	.	0.00					0	0.12	x 10^3/uL
	Lymphocyte Absolute #	.	3.39					2.5	7.5	x 10^3/uL
	Act Lymphocyte Absolute #	.	0.00							x 10^3/uL
	Monocyte Absolute #	.	0.16					0.03	0.84	x 10^3/uL
	Eosinophil Absolute #	.	0.11					0	2.4	x 10^3/uL
	Basophil Absolute #	.	0.00					0	0.2	x 10^3/uL
	Other Absolute #	.	0.00					0	0	x 10^3/uL
	Blast Absolute #		0.00							x 10^3/uL
	Promyelocyte Absolute #		0.00							x 10^3/uL
	Myelocyte Absolute #		0.00							x 10^3/uL
	Metamyelocyte Absolute #		0.00							x 10^3/uL
Differential, Vet. (VDIF)										
	Segmented Neutrophils		31							%
	Lymphocytes		64							%
	Monocytes		3							%
	Eosinophils		2							%
	Basophils									%

Table G-3a: Hematology and Clinical Chemistry for Three Holstein Bulls (Bull #1)

							RMIN	RMAX	Units
Date Section 1 Collected		**9/22/03**		**10/13/03**		**10/27/03**			
Poikilocytosis / polychromasia?		no							
Glucose	L	47.0	.	78.0	H	87.0	55	79	mg/dL
AST (GOT)	.	79.0	L	56.0	.	69.0	57	108	U/L
SDH	.	12.8	.	13.3	.	15.8	12.2	46	U/L
Total Bilirubin	.	0.1	.	0.1	.	0.1	0.1	0.4	mg/dL
Cholesterol	L	95.0	L	90.0	L	95.0	112	331	mg/dL
Total Protein	.	6.8	.	7.6	.	7.2	6.3	8.5	g/dL
Albumin	.	3.4	.	3.5	.	3.5	3.2	4.3	g/dL
Urea N	.	12.0	L	7.0	.	8.0	8	22	mg/dL
Creatinine	L	0.5	.	0.6	L	0.5	0.6	1.4	mg/dL
Phosphorous	.	9.0	.	8.3	.	8.6	4.4	9.2	mg/dL
Calcium	.	10.5	.	10.1	.	10.5	7.9	10.5	mg/dL
Sodium	.	143.0	.	150.0	.	145.0	140	151	mmol/L
Potassium	.	5.2	.	4.8	.	5.1	3.7	5.6	mmol/L
Chloride	.	100.0	H	110.0	.	102.0	100	109	mmol/L
Bicarbonate	.	25.0	.	25.0	.	29.0	22	29	mmol/L
CK	.	221.0	.	190.0	.	157.0	50	271	U/L
GGT	.	19.0	.	14.0	.	13.0	12	30	U/L
Anion Gap	H	23.0	.	20.0	.	19.0	13.6	21.6	mmol/L
Hemolysis/lipemia?		no		no		no			

Table G-3b: Hematology and Clinical Chemistry for Three Holstein Bulls (Bull #1)

							RMIN	RMAX	Units
Date Section 1 Collected		**9/22/03**		**10/13/03**		**10/27/03**			
Hemogram-Vet (VCLT)									
Red Blood Cell Count	.	7.83					5	10	x 10^6/uL
Hemoglobin	.	9.90					8	15	g/dL
Hematocrit	.	31.20					24	46	%
Mean Corpuscular Volume	L	39.80					40	60	fL
Mean Corpuscular Hemoglobin	.	12.60					11	17	pg
Mean Corpuscular Hgb Conc.	.	31.70					30	36	g/dL

Table G-3b: Hematology and Clinical Chemistry for Three Holstein Bulls (Bull #1)

								RMIN	RMAX	Units
Date Section 1 Collected			**9/22/03**		**10/13/03**		**10/27/03**			
	Red Cell Distribution Width	L	21.60					26	30	%
	Platelet Count	H	769.00					230	690	x 10^3/uL
	White Blood Cell Count	.	10.10					4	12	x 10^3/uL
	Seg. Neutrophil Absolute #	.	2.63					0.6	4	x 10^3/uL
	Banded Neutrophil Absolute #	.	0.00					0	0.12	x 10^3/uL
	Lymphocyte Absolute #	.	6.87					2.5	7.5	x 10^3/uL
	Act Lymphocyte Absolute #	.	0.00							x 10^3/uL
	Monocyte Absolute #	.	0.20					0.03	0.84	x 10^3/uL
	Eosinophil Absolute #	.	0.10					0	2.4	x 10^3/uL
	Basophil Absolute #	H	0.30					0	0.2	x 10^3/uL
	Other Absolute #	.	0.00					0	0	x 10^3/uL
	Blast Absolute #		0.00							x 10^3/uL
	Promyelocyte Abs. #		0.00							x 10^3/uL
	Myelocyte Absolute #		0.00							x 10^3/uL
	Metamyelocyte Abs. #		0.00							x 10^3/uL
Differential, Vet. (VDIF)										
	Segmented Neutrophils		26							%
	Lymphocytes		68							%
	Monocytes		2							%
	Eosinophils		1							%
	Basophils		3							%
	Poikilocytosis / polychromasia?		no							
	Glucose	L	4.0	.	76.0	H	88.0	55	79	mg/dL
	AST (GOT)	.	71.0	L	46.0	.	69.0	57	108	U/L
	SDH	L	11.2	L	7.3	.	16.7	12.2	46	U/L
	Total Bilirubin	.	0.2	.	0.1	.	0.1	0.1	0.4	mg/dL
	Cholesterol	L	88.0	L	83.0	L	86.0	112	331	mg/dL
	Total Protein	.	7.5	.	7.8	.	7.6	6.3	8.5	g/dL
	Albumin	.	3.7	.	3.4	.	3.6	3.2	4.3	g/dL
	Urea N	.	10.0	.	8.0	.	13.0	8	22	mg/dL
	Creatinine	L	0.5	L	0.5	.	0.6	0.6	1.4	mg/dL
	Phosphorous	.	7.8	.	7.3	.	8.4	4.4	9.2	mg/dL

Table G-3b: Hematology and Clinical Chemistry for Three Holstein Bulls (Bull #1)

							RMIN	RMAX	Units
Date Section 1 Collected		**9/22/03**		**10/13/03**		**10/27/03**			
Calcium	H	10.6	.	9.7	.	9.5	7.9	10.5	mg/dL
Sodium	.	142.0	.	144.0	.	145.0	140	151	mmol/L
Potassium	.	5.3	.	4.6	.	4.4	3.7	5.6	mmol/L
Chloride	.	101.0	.	104.0	.	103.0	100	109	mmol/L
Bicarbonate	.	24.0	.	27.0	.	28.0	22	29	mmol/L
CK	.	234.0	.	172.0	.	179.0	50	271	U/L
GGT	.	14.0	.	13.0	.	15.0	12	30	U/L
Anion Gap	H	22.0	.	18.0	.	18.0	13.6	21.6	mmol/L
Hemolysis / lipemia?		no		no		no			

Table G-3c: Hematology and Clinical Chemistry for Three Holstein Bulls (Bull #1)

							RMIN	RMAX	Units
Date Section 1 Collected		**9/22/03**		**10/13/03**		**10/27/03**			
Hemogram-Vet (VCLT)									
Red Blood Cell Count	.	7.41					5	10	x 10^6/uL
Hemoglobin	.	10.10					8	15	g/dL
Hematocrit	.	31.10					24	46	%
Mean Corpuscular Volume	.	41.90					40	60	fL
Mean Corpuscular Hemoglobin	.	13.70					11	17	pg
Mean Corpuscular Hgb Conc.	.	32.60					30	36	g/dL
Red Cell Distribution Width	L	21.90					26	30	%
Platelet Count	.	461.00					230	690	x 10^3/uL
White Blood Cell Count	.	4.80					4	12	x 10^3/uL
Seg. Neutrophil Absolute #	.	1.87					0.6	4	x 10^3/uL
Banded Neutrophil Absolute #	.	0.00					0	0.12	x 10^3/uL
Lymphocyte Absolute #	.	2.59					2.5	7.5	x 10^3/uL
Act Lymphocyte Abs. #	.	0.00							x 10^3/uL
Monocyte Absolute #	.	0.19					0.03	0.84	x 10^3/uL
Eosinophil Absolute #	.	0.10					0	2.4	x 10^3/uL
Basophil Absolute #	.	0.05					0	0.2	x 10^3/uL

Table G-3c: Hematology and Clinical Chemistry for Three Holstein Bulls (Bull #1)

							RMIN	RMAX	Units
Date Section 1 Collected		**9/22/03**		**10/13/03**		**10/27/03**			
Other Absolute #	.	0.00					0	0	x 10^3/uL
Blast Absolute #		0.00							x 10^3/uL
Promyelocyte Abs. #		0.00							x 10^3/uL
Myelocyte Absolute #		0.00							x 10^3/uL
Metamyelocyte Abs. #		0.00							x 10^3/uL
Differential, Vet. (VDIF)									
Segmented Neutrophils		39							%
Lymphocytes		54							%
Monocytes		4							%
Eosinophils		2							%
Basophils		1							%
Poikilocytosis / polychromasia?		no							
Glucose	L	52.0	.	76.0	H	84.0	55	79	mg/dL
AST (GOT)	.	72.0	L	53.0	.	81.0	57	108	U/L
SDH	L	9.7	L	10.1	.	18.6	12.2	46	U/L
Total Bilirubin	.	0.2	.	0.1	.	0.2	0.1	0.4	mg/dL
Cholesterol	L	85.0	L	80.0	L	87.0	112	331	mg/dL
Total Protein	.	6.6	.	7.4	.	7.4	6.3	8.5	g/dL
Albumin	.	3.6	.	3.4	.	3.3	3.2	4.3	g/dL
Urea N	.	10.0	.	8.0	.	8.0	8	22	mg/dL
Creatinine	L	0.5	.	0.6	L	0.5	0.6	1.4	mg/dL
Phosphorous	.	8.8	.	8.1	.	9.0	4.4	9.2	mg/dL
Calcium	H	10.7	.	9.7	.	10.1	7.9	10.5	mg/dL
Sodium	.	143.0	.	145.0	.	144.0	140	151	mmol/L
Potassium	.	4.9	.	4.9	.	5.1	3.7	5.6	mmol/L
Chloride	L	99.0	.	105.0	.	101.0	100	109	mmol/L
Bicarbonate	.	26.0	.	26.0	.	29.0	22	29	mmol/L
CK	.	179.0	.	134.0	.	172.0	50	271	U/L
GGT	.	15.0	.	16.0	.	17.0	12	30	U/L
Anion Gap	H	23.0	.	19.0	.	19.0	13.6	21.6	mmol/L
Hemolysis / lipemia?		no		no		no			

C. Reproductive Development and Function in Cattle Clones (Node 4)

In response to CVM's request for additional data on reproductive maturity of clones, results were submitted on semen evaluations of four post-pubertal bull clones. Semen was collected by a commercial reproduction service, from May 15, 2003 through June 19, 2003. Age of the bulls at time of sampling was not recorded. Bulls were collected three times daily, approximately every three days during the observation period. Data consists of hand-written notes provided by the technician, and includes information on semen volume, concentration, and percentage of normal sperm in samples. Sperm motility was not presented in these reports. Percent normal sperm was not assessed in all samples. A summary of the results (means, minimum and maximums) is in Table G-4. The complete table (Table G-10) is presented at the end of this Appendix. Because the original data was sent as a fax and difficult to read, some of the hand written notes may not be accurately transcribed. CVM attempted but was unable to establish contact with the service to clarify these notes.

Table G-4: Summary results of semen evaluation of four bull clones.

Clone #	Volume (ml)			Concentration ($x10^6$)			Normal Sperm (%)		
	mean	max	min	mean	max	min	mean	max	min
1	4.1	4.9	3.0	169.5	100	276	5.0	8.0	2.0
2	3.8	6.5	3.0	686.7	1870	307	51.0	71.0	25.0
3	5.0	7.9	3.5	712.1	1581	396	69.5	76.0	62.0
4	4.6	6.9	3.0	730	1649	73	63.9	80.0	54.0

Reference ranges differ somewhat, but in general normal ranges for ejaculate volume are from 4 to 15 mL, sperm concentrations from 800 to 1200 x 10^6 sperm/mL, and percent normal sperm range from 65 to 95 percent for bulls (Sorenson 1979; Beardon and Fuquay 1980; Hafez and Hafez 2000). Based on these data, unless Clone #1 was very young, he likely would have failed a breeding soundness exam, due to the very low concentration and percentage of normal sperm in the samples. Clone #2 might be considered marginal, and depending on other, unrecorded variables such as motility, and the perceived value of his genetics might have been judged acceptable. The other two clones appear to have acceptable semen, based on the limited data presented.

In 2003, Galli et al. (unpublished) presented data to CVM on three clones of a Holstein bull as a follow-up to their 2002 study on cloning (Table G-5). Scrotal circumferences of two of the clones (Clones 1 and 2) were similar to the expected range for bulls 18-24 months old (31 and 33 cm, respectively, at 22 months old vs. 32-33 cm for 18 to 24 month old bulls). Semen quality measurements on two of the clones (Clones 2 and 3) were also considered within the normal

range for young bulls, although only data on volume (5.27 and 3.35 ml) and sperm concentration (691 and 736 million/ml) were presented.

Table G-5: Scrotal circumference and semen production of clones from Galli et al. 2003 unpublished data.

	Scrotal Circumference (cm)	Collections (#)[1]	Ejaculates (#)	Average volume (ml)	Concentration ($x10^6$/ml)
Clone 1	31				
Clone 2	33	17	32	5.27	691
Clone 3		11	19	3.35	736

[1] Semen was collected at irregular intervals

Semen with ≥ 50 percent motility were frozen and thawed. Post thaw motility averaged > 40 percent. Semen from Clone # 2 resulted in a 75 percent *in vitro* fertilization rate. Semen from this clone was also used to test AI pregnancy rates on four farms. The total number of cows bred (n = 63) was small, and no contemporaneous comparator was used, so the value of this data is limited, and effects of individual farm management cannot be assessed. With these caveats in mind, the results of these tests by farm are presented in Table G-6.

Table G-6: Artificial insemination results for one clone bull (from Galli et al. 2003 unpublished data).

Farm	Cows bred	Cows pregnant	Pregnancies lost	Pregnancy rate %	Loss rate %
1	30*	22	2	73	9
2	20	10	0	50	0
3	3	1	0	33	0
4	10	8	0	80	0
total	63	41	2	65	5

*Results for two rounds of insemination were presented. It is not clear whether any of the individual cows were bred twice.

Pregnancy rates to AI in cattle vary considerably, and are affected by multiple factors, such as the ability of farm personnel to detect cows in heat, appropriate timing of insemination relative to the onset of heat, and environmental, production and nutritional factors. Studies in U.S. dairy cattle indicate that overall pregnancy rates to first AI are 40 percent or less (Lucy 2001; El-Zarkouny et al. 2004). Given the small number of cows in this study, and the lack of a contemporaneous comparator to assess the influence of farm, definitive conclusions are not possible. However, overall pregnancy rates to this bull clone do not appear worse than the U.S. average.

D. Post-pubertal Maturation in Cattle Clones (Node 5)

In response to CVM's request for data concerning this developmental node, data on two heifer clones, approximately 14 months old, was submitted to CVM (see Table G-7).
These data consist of Certificates of Veterinary Inspection, results of serological testing for Bovine Leucosis Virus (BLV), Bovine Viral Diarrhea (BVD), and hematology from the state of Wisconsin. Both heifers tested negative for BLV and BVD. According to the hematology report, both heifers had red cell distribution widths (RDW) slightly below the reference range used by the testing laboratory (22.4 and 24.0 vs. range of 26.0-30.0 percent). As discussed in Appendix E for Cyagra clones, RDW is only indicative of a health problem (anemia) when coupled with primary indicators such as low red blood cell count (RBC), hemoglobin and/or hematocrit. As all other hematology values were within the reference range, there is no evidence to indicate an underlying health problem in these animals.

Table G-7: Hematology and Clinical Chemistry for Two Holstein Heifer Clones

			Heifer #1		Heifer #2	RMIN	RMAX	Units
Date Collected			**12/10/2000**		**12/11/2001**			
Hemogram-Vet								
	Red Blood Cell Count	.	7.43	.	7.81	5	10	x 10^6/uL
	Hemoglobin	.	11	.	11.7	8	15	g/dL
	Hematocrit	.	30.6	.	32.7	24	46	%
	Mean Corpuscular Volume	.	41.2	.	41.8	40	60	fL
	Mean Corpuscular Hemoglobin	.	14.8	.	15	11	17	pg
	Mean Corpuscular Hgb Conc.	.	35.9	.	35.9	30	36	g/dL
	Red Cell Distribution Width	L	22.4	L	24	26	30	%
	Platelet Count	.	449	.	322	230	690	x 10^3/uL
	White Blood Cell Count	.	7.1	.	7.2	4	12	x 10^3/uL
	Seg. Neutrophil Absolute #	.	1.99	.	1.66	0.6	4	x 10^3/uL
	Banded Neutrophil Absolute #	.	0	.	0	0	0.12	x 10^3/uL
	Lymphocyte Absolute #	.	3.91	.	4.39	2.5	7.5	x 10^3/uL
	Act Lymphocyte Absolute #	.	0	.	0			x 10^3/uL
	Monocyte Absolute #	.	0.78	.	0.72	0.03	0.84	x 10^3/uL
	Eosinophil Absolute #	.	0.43	.	0.36	0	2.4	x 10^3/uL
	Basophil Absolute #	.	0	.	0.07	0	0.2	x 10^3/uL

Table G-7: Hematology and Clinical Chemistry for Two Holstein Heifer Clones

			Heifer #1		Heifer #2	RMIN	RMAX	Units
Date Collected			**12/10/2000**		**12/11/2001**			
	Other Absolute #	.	0	.	0	0	0	x 10^3/uL
	Blast Absolute #		0		0			x 10^3/uL
	Promyelocyte Absolute #		0		0			x 10^3/uL
	Myelocyte Absolute #		0		0			x 10^3/uL
	Metamyelocyte Absolute #s		0		0			x 10^3/uL
Differential, Vet.								
	Segmented Neutrophils		28		23			%
	Lymphocytes		55		61			%
	Monocytes		11		10			%
	Eosinophils		6		5			%
	Basophils				1			%
	Morphology		*		*			%
	Poikilocytosis / polychromasia?		no		no			

E. Summary of Cloning Studies at University of Tennessee

The original table was submitted to CVM and is presented at the end of this Appendix. The table presents data for all developmental nodes for Jersey cattle clones, including data on pregnancy losses and outcomes for surrogates. Data for progeny of clones was not included. The table represents outcomes of 46 third-trimester pregnancies of female Jersey cattle clones identified by cell line.

1. Developmental Node 1: Pregnancy and Parturition

The summary tables from the UT study indicate a total of 46 third-trimester pregnancies. Seventeen pregnancies terminated between 174 and 268 days gestation resulting from spontaneous abortion, premature delivery, or euthanasia of the surrogate dam due to advancing hydrops. In all, 27 surrogate dams experienced hydrops (27/46 or 59 percent of dams) in this cohort. This is a higher incidence of this condition than reported in previous studies or other data directly submitted to the Agency. For 10 of the 27 hydropic surrogate dams (37 percent) the condition was severe enough to require euthanasia. Twenty-five surrogate dams delivered by C-section, and an additional five cows required assistance with vaginal delivery. According to Dr. Edwards many of the C-sections were planned, due to the high incidence of hydrops.

2. Developmental Node 2: Perinatal Period

Twenty-seven calves were born alive in the UT study, of which 19 survived the critical first seven days post-partum (70 percent). Causes of perinatal death are listed in the table provided by UT, but include placental, skeletal, respiratory, renal, hepatic, and cardiac defects and lesions; aspiration of amniotic fluid; and hydrocephalus. One calf was euthanized at seven days of age. The only finding listed for this animal was blindness.

Average birth weight of these Jersey heifer clones was 31.62 kg, and ranged from a very small and non-viable calf (6.36 kg) to 45 kg. Average birth weight for conventionally bred Jersey heifers is 23-24 kg (Bonczek et al. 1992).

3. Developmental Node 3: Juvenile Period

Of the 19 calves that survived the perinatal period, 12 lived to one year of age (63 percent). One of the seven animals that died was discussed in Lawrence et al. (2005), and reviewed in Chapter V. This calf died at nine months of age from enterotoxemia due to infection with Type A *Clostridium perfringens*. The other six calves died between the ages of 41 days to eight months. Four of these calves experienced some type of gastro-intestinal problem (scouring, rumen stasis, acidosis, anorexia), with other complications (pneumonia, umbilical abscess, hepatic defects) and severe weight loss. Common pathogens such as *Mycobacterium paratuberculosis avium* and Bovine Viral Diarrhea were ruled out as potential causes of this "wasting" (Edwards teleconference). The remaining two calves were both diagnosed on necropsy with hepatic defects.

4. Developmental Node 4: Reproductive Development and Function

Information on reproductive function from the UT study is limited, and only for five females which were later euthanized for the purpose of herd reduction and one female euthanized following post partum complications (retained fetal membranes, metritis). Of these six animals, two delivered "normal" calves, but no other information on these calves (including gender) was included. The other four clones delivered stillborn calves following apparently normal pregnancies.

5. Developmental Node 5: Post Pubertal Maturation and Aging

Of the 12 calves in the UT study surviving the juvenile period, one died at 1 year of age. The heifer was noted to experience periodic scours that were unresponsive to treatment. Another heifer was euthanized at 2.5 years of age following delivery of a stillborn calf. This clone suffered complications due to retained fetal membranes (metritis) and was also noted to have had periodic scours unresponsive to treatment. According to Dr. Edwards, this clone recovered from the postpartum complications, but was euthanized due to scouring and severe weight loss. As mentioned above, five clones were euthanized at four years of age for the purposes of herd reduction. These five euthanized cows were identified as healthy at time of euthanasia. All five were noted to suckle other animals, as was the clone euthanized at 2.5 years of age. This behavior is sometimes noted in adult cattle, and may reflect how the clones were raised and weaned. The seven remaining clones are four years old, and the table indicates that their health is "within normal limits". According to Dr. Edwards, the seven remaining clones do not exhibit the aberrant suckling behavior. She attributed this to differences in how the calves were raised. The earlier group, which developed the aberrant behavior were only offered a bottle to test suckle reflex, and thereafter were fed by bucket. The latter group, from which the seven living clones derive, were fed by bottle until weaning. One of these adult clones apparently had patent urachus at one time, but is currently listed as "within normal limits". According to Dr. Edwards, the attending veterinarian noted unusually large vessels in the urachus. Another of these living clones was noted to have had an enlarged umbilicus that was surgically corrected, and is otherwise considered "within normal limits". The seven remaining clones were not bred, due to the voluntary moratorium on introducing meat or milk from clones into the food chain.

F. Perinatal Development in Swine Clones (Node 2)

Additional data was submitted by a commercial cloning company on birth weight and average daily gain (ADG) during the first three months of life as well as body temperature and heart rate of pigs during the first 2 days after birth. Breed of pigs was not identified. Data on body temperature and heart rate was available for five pigs, while birth weight and ADG were available for three of the five animals. According to the information submitted, two of the five piglets, both from the same litter and weighing 1.0 kg at birth, died within 48 hours of birth. The cause of death was not provided. The five pigs were born in two litters. The data are presented in Tables G-8 and G-9.

Table G-8: Birth Weight, Weaning Weight and Average Daily Gain for Three Swine Clones

Litter #	Piglet ID #	Birth date	BW (kg)	Age @ weighing	Period	Weight (Kg)	ADG2 (KG/day)
1	1	5/24/2002	1.2	18	17	2.7	0.06
1	1	5/24/2002	1.2	40	22	10	0.33
1	1	5/24/2002	1.2	60	20	23.3	0.67
1	1	5/24/2002	1.2	74	14	31.6	0.59
1	1	5/24/2002	1.2	94	20	50	0.92
1	2	5/24/2002	1.4	18	17	2.8	0.08
1	2	5/24/2002	1.4	40	22	8.6	0.26
1	2	5/24/2002	1.4	60	20	18.9	0.52
1	2	5/24/2002	1.4	74	14	25.8	0.49
1	2	5/24/2002	1.4	94	20	44.2	0.92
2	4	5/27/2002	1.1	15	17	3.2	0.15
2	4	5/27/2002	1.1	37	22	10.9	0.35
2	4	5/27/2002	1.1	57	20	24.6	0.69
2	4	5/27/2002	1.1	71	14	33.8	0.66
2	4	5/27/2002	1.1	91	20	51.5	0.89

Table G-9: Pulse, Respiration and Body Temperature for Five Swine Clones During First Five Days After Birth

Litter #	Pig ID #	Respiration Rate					Heart Rate					Blood Temperature				
		D1	D2	D3	D4	D5	D1	D2	D3	D4	D5	D1	D2	D3	D4	D5
1	1	48.3 +/- 3.8	36 +/- 2.3	37.9 +/- 2.1	38.9 +/- 1.7	41.3 +/- 2.8	188.6 +/- 14.4	159.8 +/- 13.8	167.4 +/- 8.1	160 +/- 6.1	176.0 +/- 5.6	99.3 +/- 1.2	101.5 +/ - 0.2	101. 9 +/- 0.3	101.2 +/- 0.4	101.3 +/- 0.3
1	2	51.8 +/- 5.7	39.1 +/- 2.2	37.3 +/- 1.7	43.7 +/- 3.7	39.5 +/- 1.8	202.3 +/- 7.3	148.6 +/- 14.6	162.3 +/- 5.9	176.7 +/- 6.7	157.4 +/- 4.8	99.2 +/- 1.0	101.8 +/- 0.4	102. 4 +/- 0.2	100.8 +/- 0.3	101.3 +/- 0.3
2	3	64.3 +/- 9.8	36.9 +/- 8.3	NA	NA	NA	162.3 +/- 10.6	173.5 +/- 2.3	NA	NA	NA	100.6 +/- 0.5	101.1 +/- 0.2	NA	NA	NA
2	4	43 +/- 3.0	34.9 +/- 1.2	NA	NA	NA	206.4 +/- 13.8	169.0 +/- 5.8	NA	NA	NA	101.0 +/- 0.5	101.1 +/- 0.3	NA	NA	NA
2	5	59.8 +/- 5.9	42.2 +/- 3.2	NA	NA	NA	172.6 +/- 13.9	180.4 +/- 6.1	NA	NA	NA	98.8 +/- 0.5	101.5 +/- 0.3	NA	NA	NA

Note: Pigs 3, 4, and 5 were from the same litter. Pigs 1 and 2 were from another litter. Pigs 3 and 5 died shortly after birth. The absence of data for pig 4 was not explained.

Birth weight and ADG vary depending on breed of swine. The breed of swine in this dataset was not reported, making interpretation of these data difficult. Likewise, interpreting respiration and heart rates in animals not typically handled is problematic, since the stress of handling tends to increase respiration and heart rates. Body temperatures of the five clones during the first two days are somewhat low; however, as noted earlier in this chapter, neonatal swine generally need supplemental heat because they lack the ability to thermoregulate. For growth, the available reference values for non-clone comparator swine and their progeny presented in the ViaGen dataset (Appendix F) is instructive. Growth rates in this dataset and the ViaGen dataset for non-clone comparators are similar. Average heart rate of day old conventional pigs was reported as 190.75 ± 36.45 bpm in one study (Foster et al. 2001).

G. Conclusions for unpublished data

Although limited in content and scope, these small datasets are consistent with the Cyagra and ViaGen datasets and with the published literature on cattle and swine clones. When considered in context with other data sources and compared to data from reference texts for conventionally bred animals, these data support the overall conclusions that cattle and swine clones generally fall within established ranges for clinical measures of health (heart rate, respiration, body

temperature, and hematology), and do not present any unique health issues. The poor semen quality of two bull clones in one small cohort cannot be explained given the limited information available on these animals. It is not unusual for bulls to fail reproductive soundness exams, and such failure may result from injury, infection, or environmental stress, among other reasons.

Sperm Characteristics of Four Clones of a Crossbred Beef Bull

Nuclear Donor (crossbred beef bull) Collection	Date	Vol (ml)	Conc X106	No. Ejac.	Dose	Exp. Yield	A Dilute	Filled	ACL	Inc	Norm	Abnormalities % Head	Droplet	Tail	Ph	Results
Clone #5	05/16/03	5.6	1253	1	35	200	112.2	198		30/	MDR's N 30%					
Clone #5	05/16/03	6.0	779	2	35	133	74.7				MDR's					
Clone #5	05/16/03	5.0	73	3	35	10	10	143		55	54	1	3	26	16	
Clone #5	05/14/03	2.9	869	1	35	72	40.3	65		50/50	MDR's N 40% ???					Disc.
Clone #5	05/14/03	3.3	1022	2	35	96	53.9	94		55	60	6	4	21	9	Disc.
Clone #5	05/14/03	5.4	697	3	35	107	60.2	100		45/45	MDR's Stressed					Disc.
Clone #5	05/22/03	6.1	576	1	35	100	56.2	99	97	55	?					Cane
Clone #5	05/22/03	5.6	415	2 & 3	35	66	371	60	56	55	68	8	4	10	10	Cane
Clone #5	05/27/03	5.5	1649	1	35	259	145.1	253		40/40	MDR's N 35%					Disc.
Clone #5	05/27/03	4.1	707	2	35	82	46.3	82		40/40	MDR's N 30% ???					Disc.
Clone #5	05/27/03	5.6	692	3	35	110	62	104	102	55	80	1	0	10	9	Cane
Clone #5	05/29/03	4.3	1198	1	35	147	82.4	153		50/50	MDR's N 30%					Disc.
Clone #5	05/29/03	3.2	342	2	35	31	17.5	27	25	50						Cane
Clone #5	05/29/03	3.6	617	3	35	63	35.5	60	56	55						Cane
Clone #5	06/02/03	5.0	1288	1	35	184	103	183	181	55						Cane
Clone #5	06/02/03	5.0	1649	2	35	235	131.9	229	227	55						Cane
Clone #5	06/02/03	5.1	502	3	25	102	57.3	96	94	60	78	2	2	10	8	Cane
Clone #5	06/05/03	3.0	697	1	25	83	46.5	80		20/25						Disc.
Clone #5	06/05/03	5.0	595	2	25	119	66.6	116	114	50	68	2	2	13	15	Cane
Clone #5	06/05/03	6.9	595	3	25	159	89	159	156	50						Cane
Clone #5	06/09/03	7.0	118	1	35	223	125	228		30/30	46	1	3	25	25	Disc.
Clone #5	06/09/03	3.5	881	2	25	183	68.9	124		40/40						Disc.
Clone #5	06/09/03	4.0	740	3	25	118	66.1	119		45/45	52	2	4	12	30	Disc.
Clone #5	06/12/03	6.2	1166	2	25	289	161.9	288		30/35						Disc.
Clone #5	06/12/03	5.6	291	3	25	65	36.5	62		30/30	73	2	0	8	17	Disc.
Clone #5	06/12/03	4.5	672	4	25	120	67.7	118	116	55	73	4	0	12	11	Cane
Clone #5	06/16/03	3.4	779	1	25	105	59.3	105		15/	MDR's					Disc.
Clone #5	06/16/03	14.5	683	2,3,4	25	397	222.4	397		50/	51	3	3	30	13	Disc.
Clone #5	06/19/03			1	Disc											
Clone #5	06/19/03	3.9	936	2		146	81.7									
Clone #5	06/19/03	4.5	817	3	25	147	82.3									
Clone #5	06/19/03	4.0	534	4	25	85	47.8									

Note: The data was transcribed from handwritten notes of the technician performing collection/evaluation of sperm