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Review

Performance of Dairy Cattle Clones and Evaluation of Their Milk Composition

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ABSTRACT

Genetic and phenotypic performance of U.S. Holstein embryo-split and nuclear-transfer clones was documented for yield and fitness traits. For cows, mean genetic superiority based on pedigree was 186 kg of milk, 9 kg of fat, and 7 kg of protein for embryo-split clones and 165, 10, and 8 kg, respectively, for nuclear-transfer clones compared with the population for the same birth year; pedigree advantage for male clones generally was slightly greater. Estimates of genetic merit that considered a clone's own performance as well as pedigree merit were slightly lower for embryo-split cows than for their full siblings for yield but not for milk composition (fat and protein percentages), mastitis resistance (somatic cell score), longevity (productive life), or cow fertility (daughter pregnancy rate); no corresponding genetic differences were found for nuclear-transfer cows or for cloned bulls regardless of clone type. For bulls, estimated genetic merit based on daughter yield was more similar for clone pairs with apparent identical genotype than for pairs from the same biotechnology but non-identical as confirmed by blood typing. Yield deviations were lower for clones than for their full siblings. Milk composition (total solids, fat, fatty acid profile, lactose, and protein) also was compared for nuclear-transfer clones (Brown Swiss, Holstein, and Holstein-Jersey cross) with non-cloned cows and literature values; no differences were found for gross chemical composition of milk. No obvious differences were evident between cloned and non-cloned animals or for the milk that they produced.

INTRODUCTION

THE CLONING TECHNOLOGIES of embryo splitting (ES) (Willadsen, 1979) and nuclear transfer (NT) (Robl et al., 1987) were introduced to dairy cattle breeding in the 1980s. For ES, an embryo is extracted from the donor and split by surgical bisections, and the demi-embryos then are transferred into recipients with reproductive cycles that have been synchronized with that of the donor. For NT, donor embryos are collected, and a nucleus (with nuclear DNA) from one of those cells is transferred to a recipient cell that has had the nucleus removed; NT embryos also can be produced from adult or fetal somatic cells (Wilmut et al., 1997). If transferred to a recipient,

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each of the NT embryos can develop into a different animal to produce a clonal family.

Although cloned dairy cattle would be expected to perform like animals from normal births, their performance had not been reviewed until recently. Natural twins are carried by the same cow, and multiple births have been shown to influence performance of cow and calves (Echternkamp and Gregory, 2002). However, most cloned animals are delivered as single births because embryos are transferred into different recipient dams; consequently, the disadvantages of multiple births can be avoided.

The genotypic agreement of NT clones also is an unresolved question because of differences in mitochondrial DNA. Little information has been recorded on the identity of the recipient cells used in the NT process, because most recipient cells have been obtained from slaughterhouses. In addition, information is not always reported that identifies which nuclei from embryo flushes, especially after multiple ovulation inducement, are associated with specific recipient cells. Consequently, determination of whether resulting clones are identical requires additional steps with some expense, such as blood and DNA typing. At present, the Animal Improvement Programs Laboratory, U.S. Department of Agriculture (Beltsville, MD), treats animals with identical genotypes as full siblings when computing genetic evaluations. Although more accurate predictions of genetic merit could be calculated for animals that are confirmed to be genetically identical, the additional resources required to confirm their genotypes may offset any possible economic gains that might result from the slight increase in genetic knowledge.

Holstein Association USA (Brattleboro, VT) began to register calves from ES in 1982 and from NT in 1989; as NT technology progressed, cell source (adult, embryo, or fetal) of the NT nuclei also was recorded. Blood typing is required by Holstein Association USA for (1) all heifers that resulted from embryo transfer and were born before July 1, 1992, (2) all bulls that resulted from embryo transfer and were born before July 1, 1988, (3) all donor dams, (4) all bulls progeny tested through AI, and (5) all NT animals. Holstein Association USA also requires that an International Embryo Transfer Society embryo recovery form be submitted for all registered animals that resulted from embryo transfer so that they can be cross-referenced to the recovery or flush from which the embryo was retrieved.

If the goal of cloning is to enhance genetic improvement, individuals selected for cloning need to be superior for genetic merit of economically important traits. A determination of the traits on which selection was based can indicate whether cloning biotechnology is providing an opportunity for genetic or economic improvement. Examination within clonal families can show whether performance is as expected from identical genotypes (i.e., the mean and variance for the progeny of clones are the same as expected for separate progeny sets of the same animal). If members of a clonal family are identical or nearly so, information from all progeny could be merged to produce a genetic evaluation for the clonal group. This shared evaluation would be more accurate than those calculated separately for each clone based on records from only their own progeny.

Interest in the impact of biotechnology on animal health and its possible impact on food safety has led to concerns about the milk that is produced by cloned cows. Typical milk composition for the domestic cow (*Bos taurus*) is 85–88% water, 3.0–4.0% crude protein, 4.6–5.2% lactose, and 3.0–5.0% fat (Walstra and Jenness, 1984). Crude protein is a measure of all sources of nitrogen and includes non-protein nitrogen, whereas true protein is a measure of only the proteins in milk; crude protein records can be converted to true protein by the formulas: true protein percentage = crude protein percentage − 0.19%; true protein weight = crude protein weight − 0.0019 (milk weight) (VanRaden and Powell, 2000). Milk composition varies by breed, lactation stage, cow age, diet, length of interval between milking, ambient temperature, disease (especially mastitis), and season of the year (Walstra and Jenness, 1984; Kaufmann and Hagemeister, 1987). Detection of differences between milk from clones and nonclones could require consideration of those effects.

The U.S. National Research Council (Committee on Defining Science-Based Concerns Associated with Products of Animal Biotechnology, 2002) established a subcommittee to identify and to prioritize science-based risks of genetically modified animals. Based on that subcommittee's report, the U.S. Food and Drug Administration (Center for Veterinary Medicine, 2001) is deciding how cloned animals should be regulated. Concern also continues about developmental aspects of clones (Young et al., 1998) that could im-

pact yield and fitness traits, and the U.S. Food and Drug Administration requested that the U.S. Department of Agriculture's Agricultural Research Service summarize the performance of dairy cattle clones throughout their lives and provide updates as additional information on clones becomes available.

HOLSTEIN CLONES IN THE UNITED STATES

Holsteins constitute over 92% of the milk-recorded dairy cattle in the United States (Powell and Sanders, 2003). Norman et al. (2002, 2004) documented numbers of U.S. ES and NT Holstein clones that were registered with Holstein Association USA by gender and birth year. A total of 2319 (1536 female, 783 male) ES clones had been registered through October 2002. Number of ES clones increased rapidly from 4 in 1982 to 246 in 1985; mean annual ES numbers were 60 females and 26 males from 1996 through 2001. A total of 215 (151 female, 64 male) NT clones had been registered through October 2002. The most female (36) and male (31) NT clones were born in 1990, the year after the first NT clone was registered. Subsequent interest in NT clones shifted toward females. Of the 60 NT clones registered between 1995 and 2002, only three were male. Among registered NT clones born between 1999 and 2003, 55 (38 females, 17 males) were cloned from adult somatic cells (20 source animals: 15 females, five males), and 21 (all females) were cloned from fetal somatic cells (three source animals) (T.J. Lawlor, Holstein Association USA, personal communication, 2003). Although the decline in numbers of male clones has diminished their potential genetic impact on the population, small numbers of male clones are sufficient to impact the population genetically. However, to date, no semen from cloned bulls has been marketed through AI in the United States (G. Doak, National Association of Animal Breeders, personal communication, 2003).

PEDIGREE MERIT

For cloned animals to enhance the population, mean genetic merit of their parents (pedigree merit) should be superior to genetic merit of the population. Norman et al. (2002, 2004) compared pedigree merit of U.S. Holstein clones with genetic merit of the U.S. Holstein population by birth year. Animals selected for cloning were slightly superior ($p \leq 0.01$) to the population genetically for yield traits. For ES females, overall mean genetic superiority of parents to the population for the same birth year was 186 kg for milk, 9 kg for fat, and 7 kg for true protein; for ES males, corresponding superiority was 254, 11, and 9 kg. For NT females, overall superiority to the population based on pedigree was 165 kg for milk, 10 kg for fat, and 8 kg for true protein; for NT males, corresponding superiority was 246, 10, and 9 kg. The small pedigree advantage for female clones of <1 standard deviation above breed mean for yield traits indicated that selection of animals to be cloned was not based exclusively on production. For NT cows from adult somatic cells, pedigree merit was lower for yield traits and higher for fitness traits compared with population means; for NT cows from fetal somatic cells, clones were genetically superior to the population for all traits (H.D. Norman, unpublished data, 2003).

Pedigree merit of U.S. Holstein clones that survived to calving and were enrolled in milk recording also was compared with genetic merit of the population (Norman et al., 2002, 2004), and a slight genetic superiority ($p \leq 0.05$) was found for the clones. For 921 ES cows with milk records, mean genetic superiority of parents to the population for the equivalent birth year was 180 kg for milk, 8 kg for fat, and 7 kg for true protein; for 172 ES bulls with genetic evaluations for yield, corresponding superiority was 314, 13, and 10 kg. For 75 NT cows with milk records, pedigree superiority to the population was 207 kg of milk, 9 kg of fat, and 7 kg of true protein; for 11 NT bulls, corresponding superiority was 335, 6, and 12 kg. No difference in genetic superiority to the milk-recorded population was evident between all female clones registered and those that survived to have lactation records.

GENETIC MERIT

Genetic merit of U.S. Holstein clones was documented by Norman et al. (2002, 2004) for yield (milk, fat, and true protein), milk composition (fat and true protein percentages), mastitis resistance (somatic cell score), longevity (productive life), and fertility (daughter pregnancy rate) (Table 1).

TABLE 1. MEAN GENETIC MERIT (PREDICTED TRANSMITTING ABILITIES) FOR U.S. MILK-RECORDED HOLSTEIN EMBRYO-SPLIT AND NUCLEAR-TRANSFER COWS AND THEIR NON-CLONED FULL SISTERS WITH RECORDS

	Embryo split		*Nuclear transfer*	
Trait	*Clones (n = 608)*	*Full sisters (n̄/clone = 1.7)*	*Clones (n = 13)*	*Full sisters (n̄/clone = 1.8)*
Milk (kg)	−37	−1[a]	−18	9
Fat (kg)	1	2[a]	−3	0
Fat (%)	0.02	0.02	−0.02	0.00
Protein (kg)	0	1[b]	2	2
Protein (%)	0.01	0.01	0.02	0.02
Somatic cell score	3.08	3.09	3.18	3.15
Productive life (months)	0.1	0.2	−0.6	−0.4
Daughter pregnancy rate (%)	0.1	0.1	−0.2	−0.1

Level of statistical significance indicated for difference between clones and full sisters ([a]$p \leq 0.01$, [b]$p \leq 0.001$); all differences between nuclear-transfer clones and full sisters were non-significant ($p > 0.05$).

All traits had been standardized to a 305 days in milk, twice daily milking, mature equivalent. Protein yield was measured as true protein; true protein weight = crude protein weight − 0.0019 (milk weight) (Van Raden and Powell, 2000).

From Norman et al. (2004).

Estimates of genetic merit for ES cows that included performance and pedigree information were slightly lower ($p \leq 0.01$) than those for their full sisters for milk, fat, and true protein yields but not significantly different ($p > 0.05$) for fat and true protein percentages, somatic cell score, productive life, or daughter pregnancy rate. No significant ($p > 0.05$) genetic differences were found for any trait between NT cows and their full sisters. For NT cows cloned from adult somatic cells, genetic evaluations for both clones and source cows were available for 9 clones from 4 source cows (H.D. Norman, unpublished data, 2003). Mean genetic merits for milk, fat, and protein yields and for daughter pregnancy rate of clones were slightly less than those of source cows; however, genetic merit for somatic cell score was the same for clones and source cows, and genetic merit for productive life was greater for clones than for the source cows.

No significant ($p > 0.05$) genetic differences were found between U.S. Holstein male clones and their full brothers (Table 2) (Norman et al., 2003). For ES bulls, estimates of genetic merit based on daughter yield were only slightly less reliable than those for their full brothers. Genetic evaluations based on daughter yield for ES bulls that appeared to have identical genotypes (99.8% probability)

TABLE 2. MEAN GENETIC MERIT (PREDICTED TRANSMITTING ABILITIES) FOR U.S. HOLSTEIN EMBRYO-SPLIT BULLS AND THEIR NON-CLONED FULL BROTHERS WITH EVALUATIONS

Trait	*Clones (n = 82)*	*Full brothers (n̄/clone = 1.3)*
Milk (kg)	−86	−78
Fat (kg)	0	1
Fat (%)	0.03	0.03
Protein (kg)	−2	−1
Protein (%)	0.01	0.01
Somatic cell score	3.2	3.1
Productive life (months)	−0.5	−0.3
Daughter pregnancy rate (%)	0.0	0.2
Daughters (no.)	139	223
Herds with daughers (no.)	77	116

All differences between clones and full brothers were non-significant ($p > 0.05$).

All traits had been standardized to a 305 days in milk, twice daily milking, mature equivalent. Protein yield was measured as true protein; true protein weight = crude protein weight − 0.0019 (milk weight) (VanRaden and Powell, 2000).

From Norman et al. (2004).

were more similar than for ES bulls with the same sire and dam but confirmed to be non-identical through blood typing; evaluation reliabilities were similar. Evaluation differences within pairs of identical ES bulls were smaller, especially for yield, than for non-identical ES bulls with the same sire and dam. Evaluation correlations for yield traits were higher for identical ES pairs: 0.87–0.92 (compared with 0.35–0.72 for non-identical ES bulls). Absolute differences in genetic evaluations for the three fitness traits (somatic cell score, productive life, and daughter pregnancy rate) also were smaller for identical ES clones than for non-identical ES bulls, and evaluation correlations were higher by 0.04–0.28. Absolute differences in evaluations should be smaller and evaluation correlations should be higher for identical ES clones than for non-identical ES bulls because the expectations for identical pairs are nearly equal to those for two independent progeny tests of the same bull. For ES bulls with confirmed identical genotypes, identical genetic merit should be assigned when calculating genetic evaluations; a recommendation for NT bulls should be deferred until more observations are available.

PHENOTYPIC PERFORMANCE

Phenotypic performance of U.S. Holstein clones also was documented by Norman et al. (2002, 2004) for standardized (305 days in milk, twice daily milking, mature equivalent) yield, mastitis resistance, and longevity traits (Table 3). Of the 1536 ES females registered since 1982, 608 had yield records through a Dairy Herd Improvement recordkeeping plan and also had non-cloned full sisters with records available for comparison. Standardized milk, fat, and true protein yields of ES cows were slightly less ($p \leq 0.01$) than those of their full sisters, but no significant ($p > 0.05$) differences were found for standardized fat and true protein percentages, somatic cell score, or productive life. Because 368 of the 608 ES cows were in herds that were different from those of their full sisters, deviations from yields of contemporaries should more accurately reflect differences between ES cows and full sisters than do standardized yields alone, which are not adjusted for herd environment. Yield deviations for milk, fat, and true protein also were slightly smaller ($p \leq 0.05$) for ES cows compared with full sisters.

Of the 151 NT females that were registered through October 2002, 13 had yield records as well as non-cloned full sisters with records available for comparison (Table 3). Nine of the 13 NT cows were in herds different from those of their 23 full sisters: six of the 13 NT cows were born within 3 months of their full sisters. No differences between NT cows and their full sisters were found for yield or fitness traits. For NT cows cloned from adult somatic cells, phenotypic per-

TABLE 3. MEANS OF STANDARDIZED TRAITS AND YIELD DEVIATIONS FOR U.S. MILK-RECORDED HOLSTEIN EMBRYO-SPLIT AND NUCLEAR-TRANSFER COWS AND THEIR NON-CLONED FULL SISTERS WITH RECORDS

	Embryo split		*Nuclear transfer*	
Trait	*Clones (n = 608)*	*Full sisters ($\bar{n}$/clone = 1.7)*	*Clones (n = 13)*	*Full sisters ($\bar{n}$/clone = 1.8)*
Standardized trait				
Milk (kg)	10,716	11,016[c]	10,456	10,715
Fat (kg)	394	402[b]	379	389
Fat (%)	3.68	3.65	3.62	3.63
Protein (kg)	320	329[c]	326	325
Protein (%)	2.99	2.99	3.11	3.03
Somatic cell score	3.1	3.1	3.6	3.0
Productive life (months)	25.7	26.4	23.1	26.7
Yield deviation from contemporaries				
Milk (kg)	−199	3[b]	−278	−305
Fat (kg)	−2	3[a]	−17	−13
Protein (kg)	−3	2[b]	−2	−7

Level of statistical significance indicated for difference between clones and full sisters ([a]$p \leq 0.05$, [b]$p \leq 0.01$, [c]$p \leq 0.001$); all differences between nuclear-transfer clones and full sisters were non-significant ($p > 0.05$).

All traits had been standardized to a 305 days in milk, twice daily milking, mature equivalent. Protein yield was measured as true protein; true protein weight = crude protein weight − 0.0019 (milk weight) (VanRaden and Powell, 2000).

From Norman et al. (2004).

formance information for both the clone and source animal was available for five clones from three source cows (H.D. Norman, unpublished data, 2003). Mean milk, fat, and protein yields and somatic cell scores of clones were similar to those of their source cow; however, yield deviations, which adjust for the herd management level, were slightly but not significantly ($p > 0.05$) lower for clones than for their source cow.

MILK COMPOSITION

Milk composition for NT cows was compared with that of non-cloned cows and literature values by Walsh et al. (2003). For biosecurity, clones and non-clones were not kept on the same farm; non-clones of approximately the same age and lactation stage on a nearby farm were used for comparison. Total solids, fat, fatty acid profile, lactose, and protein from milk during a single lactation season were analyzed for 17 NT cows and 6 non-cloned cows from five different genetic sources of three different breeds (Brown Swiss, Holstein, and Holstein–Jersey). No differences between NT and non-cloned cows were found for gross chemical composition of milk (Table 4), and all component means agreed with literature values (Kaufmann and Hagemeister, 1987) for each breed. Fatty acid content of milk was within expected ranges for both cloned and non-cloned Holsteins (Palmquist et al., 1993; DePeters et al., 1995). A difference was found between clones and non-clones for palmitic acid, but that difference may have been caused by variations in feed at the different housing locations. No differences in concentrations of individual milk proteins were found between cloned and non-cloned Holsteins. Mineral content was the most variable milk component between cloned and non-cloned Holsteins, most likely because of feed differences and variability among individual cows, but all values were consistent with the literature (Casey et al., 1995; Fox and McSweeney, 1998). Somatic cell counts from milk of clones and non-clones indicated that all cows were free of mastitis; pH values were within the reported range of ~6.5–6.8 for healthy cow's milk (Neville and Jensen, 1995).

CONCLUSION

Dairy cattle selected for cloning in the United States were slightly superior genetically for yield

TABLE 4. COMPONENT MEANS FOR MILK FROM U.S. NUCLEAR-TRANSFER AND NON-CLONED DAIRY COWS BY BREED

Breed	*Milk component*	*Clones*	*Non-clones*	*Kaufmann and Hagemeister (1987)*	*Mean for U.S. herds during 2001*
Brown Swiss	Solids (%)	13.4	13.5	13.3	—
	Fat (%)	4.3	4.5	4.1	4.0
	Protein (%)	3.6	3.2	3.6	3.4
	Lactose (%)	5.3	5.3	5.0	—
Holstein	Solids (%)	12.9	13.3	13.3	—
	Fat (%)	4.0	4.3	4.1	3.8
	Protein (%)	3.2	3.1	3.6	3.0
	Lactose (%)	5.0	5.0	5.0	—
Holstein-Jersey	Solids (%)	13.2	—	—	—
	Fat (%)	4.4	—	—	—
	Protein (%)	3.0	—	—	—
	Lactose (%)	5.0	—	—	—

For Brown Swiss, $n = 1$ for clones and non-clones. For Holsteins, $n = 13$ for clones and $n = 5$ for non-clones; one cow that was the daughter of a cloned bull was included as a clone. For Holstein-Jersey crossbreds, $n = 2$ for clones and $n = 0$ for non-clones.

All traits were based on actual percentages. Component means for U.S. herds during 2001 were reported by Wiggans (2003). Mean U.S. protein percentage was measured as true protein; all other protein percentages were measured as crude protein (percentage of milk that is true protein is lower than percentage that is crude protein by an approximate difference of 0.19%) (VanRaden and Powell, 2000).

From Walsh et al. (2003).

traits to the population. However, no obvious differences were evident between cloned and non-cloned cows or for the milk that they produced. To date, no food safety concerns related to milk from cloned cows are indicated. The U.S. Food and Drug Administration (2003) states in its draft Executive Summary of "Animal Cloning: A Risk Assessment:"

"Most clones that survive the perinatal period are normal and healthy as determined by physiological measurements, behavior, and veterinary examinations. . . . Edible products from normal, healthy clones or their progeny do not appear to pose increased food consumption risks relative to comparable products from conventional animals. Confidence in this conclusion is relatively high due to empirical evidence from bovine clones, and the consistency of empirical observations among the other species. Progeny of clones are likely to be as safe to eat as their non-clone counterparts based on underlying biological assumptions, evidence from model systems, and limited, but consistent empirical observations in the species evaluated. Additional data on the health status of progeny, and composition of milk and meat from clones and their progeny would serve to further increase the confidence in these conclusions."

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