PERFORMANCE OF HOLSTEIN CLONES IN THE UNITED STATES

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INTRODUCTION

Interest in the impact of biotechnology on food safety led the National Research Council (Board on Agriculture and Natural Resources, 2002) in the United States to establish 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) will decide how cloned animals should be regulated.

The cloning technologies of embryo splitting (Willadsen, 1979) and nuclear transfer (Robl *et al.*, 1987) were introduced to dairy cattle breeding in the 1980s. Holstein Association USA first registered calves from embryo splitting in 1982 and from nuclear transfer in 1989. Although nuclear-transfer clones are expected to have nearly identical nuclear DNA, their mitochondrial DNA will differ. Unfortunately, almost no recording has been made of the identity of recipient cells. The uncertain genetic composition of nuclear-transfer clones makes unclear whether their genetic evaluations should be the same or allowed to differ by treating clones as full siblings rather than identical animals. This study documents phenotypic and genetic performance of U.S. Holstein clones from embryo splitting and nuclear transfer for yield and fitness traits.

MATERIALS AND METHODS

Numbers of embryo-split and nuclear-transfer clones registered with Holstein Association USA were documented by gender and birth year. All nuclear transfers were from embryo rather than adult cells. For each clone group, pedigree merit (mean of predicted transmitting abilities of parents) for yield traits (milk, fat and protein) was compared with that for all U.S. Holsteins born in the same year and participating in Dairy Herd Improvement milk-recording programs. Means for each clone group were calculated for phenotypic and genetic measures of milk, fat and protein yields as well as for somatic cell score and productive life and compared with means for the population and for non-cloned full siblings.

RESULTS AND DISCUSSION

A total of 2 226 embryo-split (754 male and 1 472 female) Holstein clones were registered in the United States through 2001. Number of embryo-split clones (figure 1a) increased rapidly from 4 in 1982 to 248 in 1985. Since then, numbers have declined but only significantly ($P = 0.01$, linear and quadratic) for males. As scientific interest and subsidies in the technology waned and the procedure was commercialized, yearly numbers of embryo-split clones became more uniform (although fewer) : mean of 60 females and 26 males from 1996 to 2000.

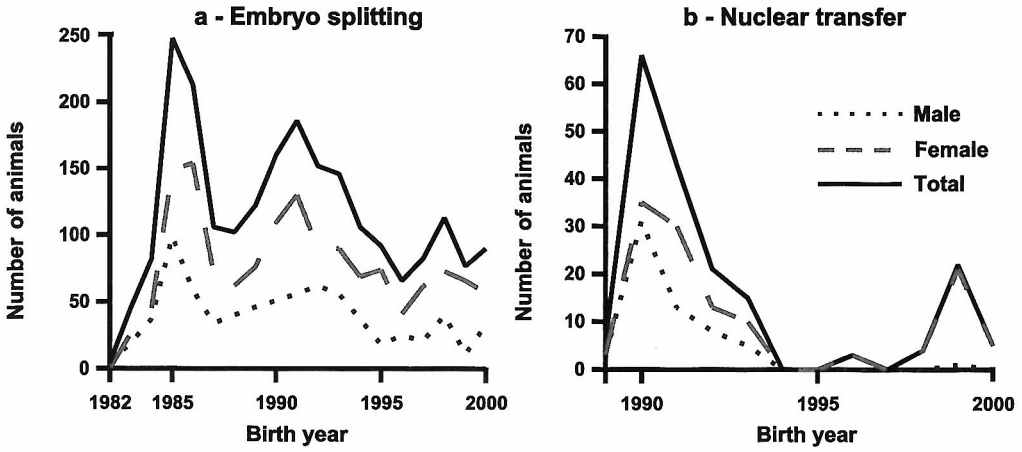

Figure 1. Numbers of registered U.S. Holsteins resulting from embryo splitting (a) or nuclear transfer (b) by gender and birth year

The larger numbers of female clones could indicate that embryos were split in conjunction with sexing or that some male clones were not of enough interest to warrant breed registration. Nevertheless, the number of male embryo-split clones is sufficient to impact the population genetically.

A total of 187 nuclear-transfer (61 male and 126 female) Holstein clones were registered through 2001. The most male (31) and female (35) clones were born in 1990, the year after the first nuclear-transfer clone was registered. Decreasing numbers of nuclear-transfer clones were linearly significant (P = 0.02 for males, P = 0.08 for females and P = 0.03 overall). The increase in nuclear-transfer clones in 1999 reflects substantial activity at one cloning site. Interest in nuclear-transfer clones has shifted toward females. Of 36 animals recorded since 1995, only 1 was male.

The decline in numbers of male clones has diminished their potential genetic impact on the population. Of 754 embryo-split male clones, only 143 had a genetic evaluation ; of those, only 22 had non-cloned full siblings. Of 61 nuclear-transfer male clones, only 10 had a genetic evaluation ; only 3 of those had non-cloned full siblings.

For clones to enhance the population genetically, their pedigree merit must be superior to that of the population (table 1). For females, mean superiority of pedigree merit of embryo-split clones compared with that of the population for the same birth year was 189 kg for milk, 8 kg for fat and 7 kg for protein ($P < 0.001$). For nuclear-transfer clones, superiority to population pedigree merit was 278, 10 and 10 kg, respectively ($P < 0.001$). The small pedigree advantage for clones of 1 standard deviation above breed mean, which is equivalent to selection of the top 38 % of the population for genetic merit for yield traits, indicates that the selection of animals to clone was not based primarily on production.

Table 1. Pedigree merit [A] for yield traits of registered U.S. Holstein females resulting from embryo splitting or nuclear transfer and the milk-recorded Holstein population by birth year

	Embryo-split clones			Nuclear-transfer clones			Population		
Birth year	Milk (kg)	Fat (kg)	Protein (kg)	Milk (kg)	Fat (kg)	Protein (kg)	Milk (kg)	Fat (kg)	Protein (kg)
1983	-487	-15	-13	. . .	. . .	. . .	-645	-22	-18
1984	-368	-11	-10	. . .	. . .	. . .	-601	-21	-17
1985	-283	-10	-10	. . .	. . .	. . .	-554	-19	-16
1986	-333	-10	-10	. . .	. . .	. . .	-506	-17	-15
1987	-322	-9	-10	. . .	. . .	. . .	-459	-15	-14
1988	-244	-3	-6	. . .	. . .	. . .	-405	-13	-12
1989	-211	-2	-5	-113	-3	-2	-344	-10	-10
1990	-79	+1	-0	-37	+4	+0	-293	-9	-9
1991	-33	+1	+0	+46	+3	+2	-233	-7	-7
1992	+48	+4	+5	+52	+5	+3	-172	-5	-5
1993	+88	+4	+5	-29	+4	+1	-114	-4	-3
1994	+88	+5	+4	. . .	. . .	. . .	-58	-2	-2
1995	+146	+6	+5	. . .	. . .	. . .	0	0	0
1996	+142	+3	+6	+396	+21	+17	+63	+2	+2
1997	+342	+10	+13	. . .	. . .	. . .	+122	+4	+4
1998	+391	+13	+14	+645	+12	+23	+172	+5	+5
1999	+432	+12	+15	+645	+12	+23	+206	+6	+7

[A] Mean of predicted transmitting abilities of parents.

Of 1 472 embryo-split female clones, 921 had yield records ; of those, 551 had non-cloned full siblings (mean of 1.5) with yield records, but 314 of the 551 clones were in 356 different herds from those of their full siblings. Differences between embryo-split clones and full siblings for standardized yields, yield deviations and predicted transmitting abilities for yield traits and productive life (table 2) were statistically significant (0.001 $\leq P \leq$ 0.03). Deviations from contemporary yields should more accurately reflect differences between clones and full siblings than do standardized yields because yield deviation accounts for herd environment and standardized yield does not. Regardless of genotypic similarity, phenotypes will vary considerably because of differences in development, environment and management (Van Vleck, 1999).

Of 126 nuclear-transfer female clones, 74 had yield records ; of those, only 11 had non-cloned full siblings (mean of 2.1) with yield records. Eight of the 11 clones were in different herds from the 15 of their full siblings. Five of the 11 clones were born within 3 mo of their full siblings. No phenotypic or genetic differences between nuclear-transfer clones and their full siblings were statistically significant ($P = 0.05$).

Table 2. Mean standardized traits and genetic evaluations for embryo-split and nuclear-transfer Holstein clones with non-cloned full siblings

	Embryo splitting		Nuclear transfer	
Trait	Clones	Full siblings	Clones	Full siblings
Standardized trait				
Milk (kg)	10 577	10 850	10 197	10 052
Fat (kg)	386	394	367	368
Fat (%)	3.66	3.62	3.61	3.66
Protein (kg)	315	324	317	306
Protein (%)	2.98	2.98	3.12	3.03
Somatic cell score	3.0	3.1	3.7	3.1
Productive life (mo)	25.6	25.3	21.4	30.3
Yield deviation from contemporaries				
Milk (kg)	-273	-93	-525	-519
Fat (kg)	-5	-1	-24	-15
Protein (kg)	-5	-0	-8	-11
Predicted transmitting ability				
Milk (kg)	-67	-34	-105	-63
Fat (kg)	0	0	-5	0
Fat (%)	0.02	0.02	-0.01	0.02
Protein (kg)	0	0	0	0
Protein (%)	0.01	0.01	0.03	0.02
Somatic cell score	3.09	3.09	3.16	3.14
Productive life (mo)	0.1	0.2	-0.4	-0.1

CONCLUSION

Numbers of clones have decreased for embryo-split males and for all nuclear-transfer clones. Animals selected for cloning were slightly superior genetically to population mean for yield traits. Yields of nuclear-transfer clones were similar to those of their non-cloned full siblings. Yields of embryo-split clones were slightly less than those of their full siblings, which may indicate an impact of the technology on performance or slightly different management of the two groups.

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