Factors Affecting Incorrect Paternity Assignment in the Israeli Holstein Population

J. I. Weller,¹ E. Feldmesser,¹ M. Golik,¹ I. Tager-Cohen,¹

R. Domochofsky,¹ O. Alus,¹ E. Ezra,² and M. Ron¹

¹Institute of Animal Sciences ARO, The Volcani Center, Bet Dagan 50250, Israel

²Israel Cattle Breeders Association, Caesaria Industrial Park, Caesaria 38900, Israel

ABSTRACT

A total of 6040 Israeli Holstein cows from 181 Kibbutz herds listed as progeny of 11 sires were genotyped for 104 microsatellites. Seventeen markers were deleted due to a frequency of erroneous genotypes >1%, leaving 160,470 valid genotypes. Conflicts between the putative sire and daughter in at least 2 markers and for at least 10% of the markers genotyped per cow were required to reject paternity. Cows that did not meet the requirements for paternity confirmation or rejection were deleted from further analysis. The frequency of rejected paternity was 11.7%. The effects of recorded sire, birth year, geographical region, herd, and inseminator on the frequency of paternity rejection were analyzed with linear and nonlinear models. Only the effects of inseminator and recorded sire were significant in all models tested that included these effects. The main causes of incorrect paternity recording appear to be inseminator recording mistakes, and possibly mistakes with respect to semen labeling at the AI institutes. Incorrect paternity recording due to multiple inseminations by different sires could explain, at most, 20% of the paternity mistakes. Instituting a system of quality control, especially at the level of the inseminator, should reduce paternity errors to no more than 8%, and increase genetic progress by at least 1%.

(Key words: paternity error, DNA microsatellites, dairy cattle, Israeli Holstein)

Abbreviation key: Ex = exclusion probability, ISAG = International Society of Animal Genetics.

INTRODUCTION

Correct pedigree information is paramount in a successful breeding program and its importance has increased with the introduction in 1990 of individual animal models for national genetic evaluations. Even

though pedigree assignments are generally not questioned, several studies have shown that the proportion of errors in sire identification varies from a few percent to as much as 22% (Christensen et al., 1982; Geldermann et al., 1986; Bovenhuis and van Arendonk, 1991; Ron et al., 1996; Visscher et al., 2002). These mistakes reduce the rate of genetic progress (Van Vleck, 1970a,b; Israel and Weller, 2000; Banos et al., 2001; Visscher et al., 2002).

Christensen et al. (1982) give the following reasons for errors in paternity recording: 1) mistakes by AI institutes in labeling semen; 2) AI technicians incorrectly identifying semen samples; 3) the insemination of cows already pregnant by a previous insemination; 4) errors when the bull's herdbook number or name is entered into the insemination record; 5) the use of natural-service bulls leading to pregnancies of previously inseminated cows which were assumed to be pregnant from the AI bull; 6) mistakes in sire identification when a cow enters the milking herd in schemes where pedigree information on milk recorded cows is obtained through the milk recording program; and 7) interchange of calves at the farm. In the Israeli Holstein population, points 5 and 6 are not relevant, because natural-service bulls are not used, and information on pedigree is not obtained through the milk-recording program.

Most previous studies that estimated the frequency of incorrect paternity were based on the analysis of, at most, several hundred cows (Ron et al., 1996; Visscher et al., 2002), and the number of animals with incorrect paternity was >100. There were only minimal attempts to analyze these samples to determine which factors were most responsible for pedigree errors (e.g., Visscher et al., 2002, compared misidentification rates in DNA derived from blood and hair samples).

Traditionally, pedigree verification in dairy cattle has been carried out using blood groups and protein polymorphisms (Stormont, 1967). Now, DNA microsatellites are the international standard system of identity verification in livestock (Bredbacka and Koskinen, 1999) and have 2 main advantages over traditional blood typing. Any sample from an individual containing

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Corresponding author: J. I. Weller; e-mail: weller@agri.huji.ac.il.

DNA can be used; hence, sampling techniques can be noninvasive and retrospective from stored tissue or semen samples. For example, Ron et al. (2003) developed and tested a method to sample cows using vaginal swabs. In addition, the accuracy is much greater than for blood group markers because microsatellites are generally polyallelic, and a virtually unlimited reservoir of markers is available (e.g., Kappes et al., 1997).

We have recently completed a complete genome scan of the Israeli Holstein population for QTL affecting economic traits by the daughter design (Weller et al., 1990; Ron et al., 2004). Eleven sire families with 6040 putative daughters were genotyped for 104 microsatellites. As a by-product of this study, paternity as listed in the herdbook could be verified or rejected for nearly all cows genotyped.

The objectives of this study were 1) to estimate the frequency of paternity misidentifications in the Israeli-Holstein population using the data collected for the genome scan, and 2) to determine the relative contributions of the factors listed by Christensen et al. (1982) to these mistakes.

MATERIALS AND METHODS

Population Analyzed and Determination of Paternity in the Herdbook

A total of 6040 cows from 181 Kibbutz (communal) herds were genotyped. Paternity was determined by the Israeli herdbook for all cows. These cows were listed as progeny of 11 sires. All but 2 cows were born between 1990 and 1997. All matings of herdbook-recorded cows were by AI, and all inseminations at Kibbutz herds were recorded.

The following algorithm determined paternity in the Israeli herdbook database: if there were a single insemination between 265 and 290 d prior to calving and the nearest insemination was at least 12 d distant, then this insemination was recorded as the effective insemination, the service sire was recorded as the sire of the calf, and gestation length was computed as calving date minus insemination date. If there were more than one insemination during this period or there was a 2nd insemination within 12 d, gestation length was not computed. If the multiple inseminations during this period were from the same sire, then this sire was recorded as the sire of calf. If the inseminations were from more than a single sire, paternity was listed as unknown.

If no inseminations were recorded between 265 and 290 d prior to parturition, but there was at least one insemination between 240 and 265 d prior to calving, the farmer was contacted, and the calf was appraised. If the farmer confirmed that a premature birth was likely, the earliest insemination during this period was

listed as the effective insemination, and paternity and gestation length were recorded accordingly. If there were no insemination between 240 and 290 d prior to calving, but there was an insemination between 290 and 300 d prior to calving, the farmer was contacted and the calf was appraised. If the farmer confirmed that a long gestation was likely, then this insemination was listed as the effective insemination and paternity and gestation length were recorded accordingly. If there were <12 d between presumed effective insemination and the next or previous insemination, but both inseminations were from the same sire, then this sire was listed as the sire of calf, but gestation length was not recorded. If the 2 inseminations were from different sires, then paternity was listed as unknown. If no insemination was recorded between 240 and 300 d prior to calving, then paternity was listed as unknown and gestation length was not recorded.

Genotyping Methods

Genotyping methods have been described in detail by Ron et al. (2004). The 73 microsatellites were run in 6 panels. Each panel of microsatellites was based on 3 to 5 multiplexed PCR reactions of fluorescent-labeled microsatellites. Microsatellites were first chosen to obtain complete coverage of the bovine genome with a maximal spacing of 50 cM between markers. Additional selection criteria were ease of scoring, mean heterozygosity, and appropriate fragment size relative to the other microsatellites included in each panel. Progeny of families with significant contrasts for specific markers were genotyped for additional markers linked to the markers with significant effects to perform interval mapping. In total, 104 markers were genotyped. The number of sires and cows genotyped per marker and the map locations of the markers genotyped, based on the Clay Center genetic map (<http://sol.marc.usda.gov/genome/cattle/cattle.html>), are given in Table 1. Markers denoted with an asterisk are included in the International Society for Animal Genetics (ISAG) standard panel for pedigree confirmation (Bredbacka and Koskinen, 1999). Eight of the 11 markers included in the panel were genotyped in the current study. There were a total of 188,717 valid cow genotypes and 682 sire genotypes. Thus, the mean number of genotypes per marker was 1815 for cows and 6.6 for sires.

Edits on Genotypes

The rules of Mendelian inheritance require that each parent and progeny should share at least one common allele. Discrepancies between the cow and putative sire genotypes may be due to mutation, errors in genotyp-

Table 1. Marker locations, number of sires and cows genotyped, single conflicts per marker, frequency of single conflicts, and deviations from expected heterozygote frequency.

Marker ¹	BTA ²	Position ³	Number of		Single conflicts		Heterozygosity obs – ex ⁴
			Sires	Cows	Number	Frequency	
BM4307	1	35.2	9	1868	11	0.006	–0.03
INRA049	1	67.5	9	2221	13	0.006	–0.04
BM1824*	1	108.6	9	2353	1	0.000	0.00
ETH121	2	34.0	10	2255	1	0.000	–0.00
BMS1126	2	56.0	10	3050	2	0.001	0.04
RMN041	2	69.0	3	1039	17	0.016	0.03
BMS1866	2	82.7	2	667	4	0.006	–0.01
BMS1987	2	98.2	2	1093	4	0.004	–0.18
BMS2519	2	101.5	3	1029	18	0.017	–0.01
BM2113*	2	106.2	9	2960	14	0.005	0.03
IDVGA37	2	108.2	1	670	1	0.001	0.03
IDVGA2	2	117.8	2	602	8	0.013	–0.02
FCB11	2	120.4	3	1372	13	0.009	0.02
UWCA7	3	19.9	9	2513	35	0.014	–0.19
INRA003	3	59.5	11	3122	1	0.000	0.06
HUJ1177	3	87.4	10	2872	11	0.004	–0.01
INRA200	3	109.7	8	2272	24	0.011	–0.03
RM188	4	24.7	11	2952	5	0.002	–0.00
BM6458	4	68.3	10	2309	24	0.010	–0.03
BP1	5	19.0	10	2746	12	0.004	–0.03
ETH10*	5	70.0	9	2363	0	0.000	0.02
ILSTS93	6	0.0	3	1336	3	0.002	0.00
INRA133	6	8.2	2	711	4	0.006	–0.17
ILSTS90	6	11.8	1	543	4	0.007	0.04
BMS1329	6	35.5	2	658	5	0.008	0.06
BMS2508	6	44.2	2	898	0	0.000	–0.00
BM143	6	49.4	9	2633	1	0.000	0.01
BMS518	6	55.2	2	1113	0	0.000	0.01
BMS483	6	64.0	3	1051	0	0.000	–0.02
BMS360	6	66.5	3	1308	10	0.008	0.09
BM415	6	76.3	9	2556	1	0.000	–0.01
BM8124	6	94.2	1	573	2	0.003	0.09
BMS739	6	113.4	3	1282	2	0.002	0.02
BM7160	7	0.0	2	440	6	0.014	0.10
BMS713	7	15.2	2	421	2	0.005	0.01
RM006	7	22.1	7	2207	0	0.000	0.04
BM6105	7	35.7	1	423	4	0.009	0.15
TGLA303	7	38.5	1	439	1	0.002	0.08
BM7247	7	58.0	1	414	1	0.002	0.06
UWCA20	7	59.9	7	2550	5	0.002	0.02
BMS2840	7	64.3	1	322	0	0.000	0.13
INRA112	7	72.1	1	324	2	0.006	0.07
BMS792	7	76.9	1	343	0	0.000	0.03
BMS1331	7	91.1	1	336	0	0.000	–0.00
ILSTS006	7	116.0	11	2852	2	0.001	0.03
BMS1591	8	28.1	10	2753	33	0.012	–0.03
BMS1341	8	51.4	11	2971	3	0.001	0.01
BM711	8	83.6	7	2013	2	0.001	0.00
BM757	9	0.6	7	2163	0	0.000	0.04
ETH225*	9	8.1	7	2143	1	0.000	0.01
UWCA9	9	44.9	3	478	0	0.000	0.07
BMS1290	9	59.0	11	3170	3	0.001	0.01
BMS2295	9	91.5	9	1843	21	0.011	–0.03
BRN	10	29.2	10	2221	33	0.015	0.02
BMS1318	10	73.0	9	2375	1	0.000	0.02
RM096	11	31.0	8	2133	0	0.000	0.02
BM8118	11	72.4	9	2816	11	0.004	–0.04
HEL13	11	114.0	9	1753	4	0.002	–0.01
IDVGA57	12	29.9	10	2730	4	0.001	–0.09
BM860	12	49.4	7	1824	0	0.000	0.04
BM4028	12	79.7	7	2349	0	0.000	0.03
BMC1222	13	20.0	10	2287	12	0.005	–0.16
BMS1352	13	31.0	7	2147	2	0.001	0.01

Continued

Table 1 (Continued). Marker locations, number of sires and cows genotyped, single conflicts per marker, frequency of single conflicts, and deviations from expected heterozygote frequency.

Marker ¹	BTA ²	Position ³	Number of		Single conflicts		Heterozygosity obs – ex ⁴
			Sires	Cows	Number	Frequency	
BMS995	13	84.1	11	2899	2	0.001	0.03
ILSTS039	14	20.0 ⁵	7	1582	0	0.000	0.02
CSSM66	14	33.0 ⁵	7	2066	0	0.000	0.02
BM4305	14	66.4	7	1511	8	0.005	–0.02
BMS2684	15	34.9	9	1226	6	0.005	–0.05
HBB	15	39.6	7	2066	0	0.000	0.03
SPS115*	15	70.0	8	1978	1	0.001	–0.00
TGLA245	16	6.5	5	428	9	0.021	–0.05
IDVGA68	16	47.4	9	2636	11	0.004	0.04
INRA13	16	88.0	3	449	0	0.000	–0.02
BMS2220	17	16.0	8	1313	5	0.004	–0.06
IDVGA40	17	67.0	9	2662	10	0.004	–0.01
BM7109	18	49.6	10	2628	8	0.003	–0.01
TGLA227*	18	84.7	9	2205	1	0.000	0.02
BM745	19	15.9	8	2271	23	0.010	–0.02
BP20	19	46.5	7	2113	0	0.000	–0.01
IDVGA48	19	70.7	9	1928	36	0.019	–0.40
BM1225	20	8.0	7	2264	5	0.002	–0.00
TGLA126*	20	31.2	9	2376	1	0.000	0.05
BM5004	20	64.3	9	1934	10	0.005	–0.03
ETH131	21	32.3	6	1665	2	0.001	–0.02
TGLA122*	21	67.3	9	2346	0	0.000	0.01
CSSM41	22	46.3	7	1984	3	0.002	–0.07
CYP21	23	36.0	7	2155	0	0.000	0.01
BM7151	24	6.0	9	2188	28	0.013	0.02
BMS466	24	46.0	9	2477	10	0.004	–0.11
ILSTS102	25	6.5	10	2890	11	0.004	0.03
BMS1353	25	45.3	9	2524	8	0.003	–0.00
BMS651	26	2.5	10	2758	15	0.005	–0.01
BM4505	26	40.0	10	2106	5	0.002	–0.01
BMS2168	27	0.0	11	3101	5	0.002	–0.02
BMS641	27	14.3	1	562	1	0.002	0.09
BMS689	27	34.1	11	3826	29	0.008	0.00
CSSM36	27	39.8	2	914	10	0.011	0.01
BM1857	27	52.7	2	915	10	0.011	0.00
INRA027	27	58.4	1	603	2	0.003	0.04
BM203	27	64.1	1	491	2	0.004	0.06
BMC6020	28	2.0	5	929	7	0.008	–0.15
IDVGA43	28	27.9	9	2582	47	0.018	–0.24
ARO26	29	11.0	7	2037	0	0.000	0.02
BMC6004	29	57.9	10	2899	1	0.000	–0.00
Means			6.55	1815	7.01	0.004	–0.004

¹Markers denoted with an asterisk are included in the International Society for Animal Genetics (ISAG) standard panel for pedigree confirmation.

²*Bos taurus* autosome.

³Marker locations are given according to the Clay Center genetic map (<http://sol.marc.usda.gov/genome/cattle/cattle.html>), unless otherwise indicated.

⁴Difference between the observed and expected frequencies of heterozygotes, based on the Hardy-Weinberg equilibrium.

⁵Riquet et al. (1999).

ing, “null alleles,” or errors in paternity recording. As noted, the Israeli Holstein genome scan included more than 180,000 individual marker genotypes, and a data-set of this magnitude should contain all 4 factors.

Various studies have reported on the presence of null alleles for specific microsatellites (e.g., Petersen and Bendixen, 2000). If one allele fails to amplify, the individual appears to be a homozygote for this marker, even

though it is in fact a heterozygote. Progeny of a sire heterozygous for a null allele that receive the null allele will appear to be homozygous for their maternal allele. If the maternal allele is different from the sire’s observed allele then no common allele will be observed between the sire and his progeny, and paternity will be erroneously rejected. A sire was declared to be heterozygous for a null allele if the following 3 criteria

were met: 1) the sire was observed to be apparently homozygous for the microsatellite, 2) there was a preponderance of observed homozygous progeny of the sire as compared with the expected frequency by Hardy-Weinberg equilibrium as estimated from the observed allelic frequencies; and 3) more than 10% of the recorded progeny were homozygous for alleles other than the observed putative paternal allele.

The difference between the observed and expected frequency of heterozygotes, based on Hardy-Weinberg equilibrium was computed for each marker, and is also listed in Table 1. The expected frequency of heterozygotes for marker j , HEX_j , was computed as follows:

$$HEX_j = \frac{K_j}{k_j} \sum_{k=1}^{k_j} p_{jk} \times (1 - p_{jk})$$

where p_{jk} = the frequency of allele k of marker j , and K_j is the total number of alleles for marker j . The mean frequency of heterozygotes over all markers was 0.68, and the difference between the observed and expected heterozygosity ranged from -0.40 to 0.15 , with a mean of -0.004 .

Based on these criteria, 3 markers were found with null alleles: CSSM41, IDVGA43, and IDVGA48. One sire was determined to be heterozygous for a null allele for CSSM41, and 3 sires were heterozygous for null alleles for IDVGA43 and IDVGA48. In these cases, a conflict between the genotype of the daughter and the putative sire was declared only if the daughter was observed to be heterozygous for the marker. The difference between the observed and expected heterozygosity for these markers were -0.07 , -0.24 , and -0.40 , respectively. Thus, for these 3 markers, there were fewer heterozygotes than expected by the Hardy-Weinberg equilibrium. Considering all 104 markers, IDVGA43 and IDVGA48 had the highest deviations from the expected frequency of heterozygotes.

If the cow and the putative sire are genotyped for many markers, and paternity recording is incorrect, then it is very unlikely that only a single discrepancy should be observed. These single discrepancies may be due either to mutations or genotyping mistakes. Mutation rates for microsatellites are on the order of 10^{-4} (Crawford and Cuthbertson, 1996), whereas genotyping errors are on the order of 1%. When results of 33 laboratories on 40 DNA samples were compared, identical results were not obtained for any of the 9 basic ISAG markers, and 2 laboratories differed in more than one allele (Glowatzki-Mullis and Muntwyler, 2002). Thus, the most likely explanation for single discrepancies for cows genotyped for many markers is that paternity was correctly recorded and the single discrepancy is a geno-

typing mistake. Of the 104 markers genotyped, some were more prone to genotyping mistakes. The frequencies of cows with single discrepancies among all cows genotyped for each marker were computed for all 104 markers, and are listed in Table 1. Markers with a frequency of single discrepancies $>1\%$ were considered to be "problematic" and were deleted from further analysis. Seventeen markers met this criterion, including 2 of the markers with null alleles, leaving 87 markers with 160,470 valid genotypes, and a mean of 1844 genotypes per marker.

Calculation of Exclusion Probabilities

Exclusion probability for cow i , Ex_i , was calculated as follows (Ron et al., 1996):

$$Ex_i = 1 - \prod_{j=1}^J [1 - (1 - p_{ij})^{**2}]$$

where p_{ij} = sum of the frequencies of the 2 alleles of cow i for marker j , and $\prod[y]$ represents the product of $[y]$ from 1 through J , where J is the number of markers for which cow i and her putative sire were genotyped. For those markers for which the cow was homozygous, p_{ij} = the frequency of the allele of cow i for marker j . Exclusion probabilities were computed for all cows with less than 2 markers in conflict with the genotype of the putative sire.

Note that if p_{ij} are approximately equal for all loci, then $1 - Ex_i$ should be a multiplicative function of the number of markers genotyped. Thus, $\log(1 - Ex_i)$ should be an approximately linear function of the number of markers. Correlations among Ex_i , $\log(1 - Ex_i)$ and the number of markers genotyped per cow were computed. The regression of $\log(1 - Ex_i)$ on the number of markers genotyped was also computed.

Confirmation of Paternity by Genotypes

Three categories were determined with respect to paternity confirmation: confirmation, rejection, or undetermined. If no discrepancies were found between the putative daughter and sire genotypes, then paternity recording was declared confirmed if the exclusion probability was >0.9 . If no discrepancies were found, but the exclusion probability was <0.9 , then paternity confirmation was declared undetermined. If 2 or more discrepancies were found among the first 20 valid genotypes then paternity was rejected, and generally no further genotyping was performed. If a single discrepancy was found, then genotyping was generally continued. Paternity was declared confirmed if the cow was

Table 2. Number of cows by the criteria used for paternity confirmation.

Number of			Frequency of discrepancies	Exclusion probability	Paternity	
Cows	Genotypes	Discrepancies			Status	No. cows
4620	2–64	0	0	>0.9	Confirmed	4929
309	20–64	1	<0.1	>0.9	Confirmed	
210	1–8	0	0	≤0.9	Undetermined	457
133	1–19	1	0.05–1	...	Undetermined	
114	20–61	2–4	<0.1	...	Undetermined	
654	3–62	2–11	≥0.1	...	Rejected	654
Total						6040

genotyped for at least 20 markers and no further discrepancies were found. In this case, the single discrepancy was assumed to be a mistake or possible mutation. If there were a single discrepancy, but there were <20 valid genotypes, then paternity was declared undetermined. If the cow was genotyped for ≥20 markers, paternity was rejected if there were a discrepancy between the cow and putative sire genotypes in at least 3 markers and ≥10% of the microsatellites. Finally, paternity was declared undetermined if discrepancies were found for ≥2 microsatellites, but <10% of all the markers genotyped. The number of cows in each category is summarized in Table 2. There were 457 cows with undetermined paternity. These cows were deleted from all further analyses, leaving 5583 cows; 4929 cows with confirmed paternity and 654 cows with rejected paternity. Preliminary analyses showed that minor changes in the criteria used had very small effects on the number of cows assigned to each paternity status.

Analysis of Factors Affecting Paternity Misidentification

As noted in the Introduction, the main objective of genotyping was to detect segregating QTL using the daughter design. Therefore, in the early stages of analysis, the genotypes of 96 cows with rejected paternity were not retained. Thus, of the 654 cows with rejected paternity, there were 558 with genotype data on the individual markers. The number of total genotypes, genotypes in conflict with the putative sire, and the frequency of genotypes with conflicts were analyzed. The 96 cows with rejected paternity, but without genotype data, were included in all analyses that did not require specific genotype information.

To determine the possible effect of switching newborn calves on paternity errors, the dates with multiple calvings were determined for each herd. The frequency of misidentified paternity was then computed separately for dates with single and multiple calvings in each herd.

Significance of the difference in the frequency of misidentified paternity was tested by χ^2 analysis.

To determine the effect of the inseminator responsible for the mating that resulted in each cow genotyped, the dates of the insemination records of the dam of each cow were compared with the cows' birthdates. Of the 5583 cows with either confirmed or rejected paternity, there were only 39 for which the dam was not recorded. A possible match between an insemination record and the cow's birth record was declared if the insemination date was between 240 and 300 d prior to parturition. This resulted in some multiple matches, but there were only 17 cows for which a matching insemination record was not found, leaving 5526 cows with at least one possible matching insemination record for their dam. Most of the cases of missing insemination records were apparently due to dams of genotyped cows that changed herds.

For cows with multiple matches, only a single insemination record was retained. First priority was given to insemination records with recorded gestation lengths, even if a negative pregnancy check were recorded (Weller and Ezra, 1997). There were 8 cases in which both gestation length and a negative pregnancy check were recorded. The negative pregnancy check was assumed to be a mistake for these records. If no gestation length were recorded in the herdbook, then priority was given to records with positive pregnancy checks. If gestation length was not recorded, and there were multiple records with positive pregnancy checks, or none of the records had positive pregnancy checks, then the insemination resulting in the longest pregnancy interval up to 300 d was retained. There remained a single cow for which gestation length was not recorded and the pregnancy check was negative on the only matching insemination record. The service sire and sire of the genotyped cows were the same and paternity was confirmed based on the genetic markers. We therefore assumed that for this cow the negative pregnancy check was a mistake. After these edits, there were 2 cows

for which the service sire on the insemination record differed from the recorded sire of the genotyped cow. Paternity was confirmed for both cows, but neither gestation length nor a pregnancy check was recorded. These records were also retained. The putative gestation length was computed for each cow with a matching insemination record. The relationship between the recorded gestation period and the frequency of rejected paternity was determined by a χ^2 test. Gestation period was divided into 6 categories of equal length.

The geographical region for each herd was determined and matched to the cow record. Seven geographical regions based chiefly on climate are defined in the Israeli Holstein herdbook. There were at least 318 cows from all the geographical regions, except for the Arava Valley. There was only one cow from a herd located in this region.

The effects of putative sire, birth year, herd, region, and inseminator on the frequency of rejected paternity were tested by the following linear model, termed model I:

$$P_{ijklmn} = S_i + B_j + R_k + H_{kl} + T_m + e_{ijklmn}$$

where P_{ijklmn} is the paternity confirmation score (0 for rejected paternity and 1 for confirmed paternity) of cow n , putative daughter of sire i , born in birth year j , from herd l of region k , produced by insemination technician m ; S_i is the effect of sire i ; B_j is the effect of birth year j ; R_k is the effect of geographical region k ; H_{kl} is the effect of herd l within region k ; T_m is the effect of insemination technician m ; and e_{ijklmn} is the random residual. Birth years, herds, regions, and inseminators with less than 10 records were deleted, leaving 5320 cows. Records from 1 region, 2 birth years, 7 inseminators, and 68 herds were deleted. Herds were nested within region; all other effects were factorial.

In model II, the class effect of birth year was replaced by the regression effect of birth date. Least square means were not estimable by model I or II because of confounding between herds and inseminators. In general, each inseminator worked an area consisting of several herds. Least square means were computed from 2 additional models based on model I. In model III, the herd effect was deleted, and in model IV, the inseminator effect was deleted. Model V included only the effects that were significant in model I ($P < 0.05$). These models were compared based on coefficients of determination and F -values derived from the "type-III" sums of squares (SAS Inst., Inc., Cary, NC). Finally, in model VI, paternity confirmation score was analyzed by PROC CATMOD (SAS) using maximum likelihood estimation with generalized logits as the response function. Model VI included the effects of region, sire, and birth year.

Figure 1. Number of cows with confirmed paternity by number of markers genotyped.

Significance could not be obtained for models that included either the inseminator or herd effect because of the large number of empty cells.

RESULTS

The total frequency of rejected paternity determinations was 11.7%. The number of cows with confirmed paternity by number of markers genotyped is given in Figure 1. The number of markers for cows with confirmed paternity ranged from 2 to 64 with a mean of 29.4. $\log_{10}(1 - Ex_i)$ for cows with exclusion probabilities >0.9 ranged from -1 to -26.6 . The correlation between $\log_{10}(1 - Ex_i)$ and number of markers per cow was -0.96 . The regression of number of markers on $\log_{10}(1 - Ex_i)$ was -0.361 , with a standard error of 0.002. The y-intercept was -0.088 , with a standard error of 0.050. Thus, on the average, 2.5 markers are required to obtain an exclusion probability of 0.9, and 5.3 markers are required to obtain an exclusion probability of 0.99. The number of markers for cows with exclusion probabilities <0.9 ranged from 1 to 8 with a mean of 3.2. This value is slightly greater than the value of 2.5 predicted by the regression equation.

The number of cows with rejected paternity for the number of markers genotyped and the number of markers in conflict with their sire's genotype are given in Figure 2. There were 7 cows with rejected paternity and >40 markers that were not included in the figure. The number of markers genotyped per cow for the cows with rejected paternity ranged from 3 to 62, with a mean of 16.8. The number of markers in conflict ranged from 2 to 16, with a mean of 4.5. As noted, genotyping of cows with rejected paternity was generally discontinued once at least 2 markers in conflict with the sire

Figure 2. Number of cows with rejected paternity by number of markers genotyped (solid bars), and number of markers in conflict with their sire's genotype (hatched bars). An additional 7 cows genotyped for >40 markers are not shown.

genotype were found. However, the markers were run in panels of up to 15 markers, and all markers in the panel were analyzed jointly. The number of cows with rejected paternity by the fraction of markers in conflict with the sire is given in Figure 3. The fraction ranged from 0.1 to 1.0, with a mean of 0.30. As noted in the methods, a frequency of at least 0.1 marker discrepancies was required to reject paternity.

The number of levels, records, F -values, and coefficients of determination (R^2) in the linear model analyses are given in Table 3. For the categorical model analysis, χ^2 values were computed instead of F -values, and

Figure 3. Number of cows with rejected paternity by the fraction of markers in conflict with the sire.

R^2 was not computed. The R^2 at 0.132 was highest for model I. Although the effects of region and herd were not significant in model I, both effects were highly significant in model IV, which did not include the inseminator effect. As noted previously, the herd and inseminator effects are highly confounded because most inseminators worked a specific region consisting of several herds. The effect of region was also not significant in model III, which included the inseminator effect, but not the herd effect. The effects of sire and birth year were also strongly confounded. Some sires were used chiefly in the early years, whereas other sires were used in the later years. The sire effect was highly significant in all models, whereas the class effect of birth year was only marginally significant in the linear models. The linear effect of birth date was not significant in model II, but the class effect of birth year was significant ($P = 0.0025$) in the categorical model that also included the sire effect. The R^2 for model V, which included only the effects significant in model I, was 0.110, as compared with 0.132 for the complete model. The 3 effects included in the categorical model analysis were all significant at $P < 0.005$.

The number of cows genotyped, the number of cows with rejected paternity, the frequency of rejected paternity, and the model III least square means by recorded sire are given in Table 4. Frequency of paternity rejection was lowest for sire 3241 (7%), and 3 times higher (22%) for sire 3099. The high frequency of mistakes for sire 3099, double the overall average, is surprising, and may be due to a mistake in semen labeling at the AI institute. This sire was used chiefly in the earlier years included in the analysis, whereas 3241 was used chiefly in the later years. Least square means were generally lower than the simple means for the sires with lower numbers that were used earlier, and higher than the simple means for the sires that were used later. Standard errors of the least square means ranged from 0.017 to 0.026.

The number of cows genotyped, the number of cows with rejected paternity, the frequency of rejected paternity, and the model III least square means by birth year are given in Table 5. The frequency of rejected paternity ranged from 19% in 1990 to 6% in 1997. Although a clear trend of reduction in the rate of rejected paternity is evident in the simple means, this is not the case for the least square means. Although the lowest least square mean was for 1997, the last birth year included in the analysis, the highest least square mean, was for 1994, in the middle of the distribution. Standard errors ranged from 0.013 to 0.045. As noted previously, the linear effect of birth date was not significant.

The number of cows genotyped, the number of cows with rejected paternity, the frequency of rejected pater-

Table 3. The number of levels, records, *F*-values, and coefficients of determination (R^2) in the linear model analyses (5320 cows).

Model	Model type	Factor	Levels	<i>F</i> -values ¹	Significance	R^2
I	Linear	Inseminator	50	4.38	<0.0001	0.132
		Region	6	0.59	NS ²	
		Herd	123	1.08	NS	
		Sire	11	3.34	0.0002	
		Birth year	8	2.05	0.0451	
II	Linear	Inseminator	50	4.38	<0.0001	0.130
		Region	6	0.49	NS	
		Herd	123	1.10	NS	
		Sire	11	3.11	0.0006	
		Birth date ³		0.86	NS	
III	Linear	Inseminator	50	10.24	<0.0001	0.111
		Region	6	0.94	NS	
		Sire	11	3.64	<0.0001	
		Birth year	8	2.49	0.0150	
IV	Linear	Region	6	10.90	<0.0001	0.097
		Herd	123	3.46	<0.0001	
		Sire	11	3.21	0.0004	
		Birth year	8	2.19	0.0325	
V	Linear	Inseminator	50	11.79	<0.0001	0.110
		Sire	11	3.70	<0.0001	
		Birth year	8	2.42	0.0179	
VI	Logistic ⁴	Region	6	68	<0.0001	
		Sire	11	36	<0.0001	
		Birth year	8	22	0.0025	

¹For type III sums of squares.²Not significant.³Number of categories is not listed because birth date was a linear regression effect.⁴The χ^2 values for the maximum likelihood estimates are given instead of *F*-values. The R^2 values are not computed for the logistic analysis.

nity, and the model III least square means by geographical region are given in Table 6. The frequency of rejected paternity was lowest in the Jordan Valley region (5%) and highest in "other areas" (15%). Similar to both sire and birth year, the frequency of incorrect paternity in the worst level was 3 times higher than in the best level. Differences among the least square means were smaller than the difference among the simple means.

Table 4. The number of cows genotyped, the number of cows with rejected paternity, and the frequency of rejected paternity by recorded sire.

Sire	Number of cows		Frequency of rejected paternity	Least square means ¹
	Genotyped	Rejected paternity		
2278	748	94	0.126	0.104
2283	414	53	0.128	0.120
2357	563	59	0.105	0.062
3070	729	94	0.129	0.099
3089	542	53	0.098	0.080
3099	275	61	0.222	0.180
3208	530	40	0.075	0.084
3212	532	74	0.126	0.118
3241	572	40	0.070	0.087
3258	346	40	0.116	0.153
3274	332	46	0.139	0.150
Total	5583	654	0.117	

¹By Model III, 5320 cows were included in the analysis.

The number of cows genotyped, the number of cows with rejected paternity, the frequency of rejected paternity, and the model IV least square means of 5 herds with the highest proportion of rejected paternity, and the 5 herds with the lowest proportions of rejected paternity and a minimum size of 50 cows are given in Table 7. The proportion of rejected paternity ranged from 32 to 74% for the 5 herds with the highest proportion of rejected paternity. These 5 herds included <4%

Table 5. The number of cows genotyped, the number of cows with rejected paternity, and the frequency of rejected paternity by birth year.

Birth year	Number of cows		Frequency of rejected paternity	Least square means ¹
	Genotyped	Rejected paternity		
1990	57	11	0.193	0.143
1991	407	60	0.147	0.129
1992	1036	121	0.117	0.131
1993	903	109	0.121	0.116
1994	1313	174	0.133	0.153
1995	1000	90	0.090	0.129
1996	766	83	0.108	0.088
1997	99	6	0.061	0.009
Total ²	5581	654	0.117	

¹By Model III, 5320 cows were included in the analysis.²Two cows were born outside the range of 1990 to 1997.

Table 6. The number of cows genotyped, the number of cows with rejected paternity, and the frequency of rejected paternity by geographical region.

Region	Number of cows		Frequency of means	Least square means ¹
	Genotyped	Rejected paternity		
Mountains	123	15	0.122	0.110
Jordan Valley	996	51	0.051	0.084
Jezrael Valley	318	29	0.091	0.107
Negev	955	139	0.146	0.119
Other regions	1063	160	0.151	0.139
Coastal plain	2127	260	0.122	0.117
Total ²	5582	654	0.117	0.110

¹By Model III, 5320 cows were included in the analysis.

²One additional cow was from the Arava region.

of all the cows genotyped, but included 14% of the cows with rejected paternity. The proportion of rejected paternity ranged from 0 to 3.5% for the 5 herds with the lowest proportion of rejected paternity and minimum size of 50 recorded cows. The least square means were very similar to the simple means for the herds included in Table 7. There were 13 herds with at least 10 genotyped cows and no cases of rejected paternity. Ten herds had least square means <0, considering all herds included in the linear model analyses, but all of these herds had relatively few records.

Of the 5583 cows with paternity either confirmed or rejected, 3404 (61%) were born on dates with multiple calvings in the herd, and 2179 (39%) were born on dates with a single calving. The rate of paternity errors were 10.8% for dates with single births, and 12.3% for dates with multiple births. Although this difference of 1.5% is in the expected direction, the χ^2 probability was 0.10. This supports the conclusion from model I that the herd effect is not a significant factor in determining the rate of paternity misidentification.

Mean computed gestation length was 275.7 d, with a standard deviation of 5.1 d. The number of cows genotyped, the number of cows with rejected paternity, and the frequency of rejected paternity by gestation length category are given in Table 8. Frequency of rejection was lowest for the "normal" gestation lengths, 270 to 289 d, and higher for the cows of either short or long gestations. The relationship between recorded gestation period and frequency of rejected paternity was tested by χ^2 with the 6 categories as listed in Table 8, and with the first 2 categories combined to obtain the minimum required expected cell values. In both cases the χ^2 values were significant at $P < 0.005$. Thus, if the computed gestation length is deviant, the probability of incorrect paternity is increased.

Of the cows with rejected paternity and identified inseminator, there were 131 cows for which a match

Table 7. The number of cows genotyped, the number of cows with rejected paternity, and the frequency of rejected paternity for the 5 herds with the highest fraction of rejected paternity, and the 5 herds with the lowest fractions of rejected paternity and at least 50 cows.

Herd ¹	Number of cows		Frequency of means	Least square means ¹
	Genotyped	Rejected paternity		
435000	38	28	0.736	0.789
707000	13	6	0.462	0.473
274000	78	29	0.372	0.333
370000	61	20	0.328	0.319
424000	19	6	0.316	0.287
334000	115	4	0.035	0.048
262000	58	2	0.034	0.034
176000	59	2	0.034	0.019
590000	78	2	0.026	0.019
188000	56	0	0.000	0.015

¹By Model IV.

was found to 2 or more insemination records and with at least 2 different service sires listed. Thus incorrect determination of the sire due to multiple inseminations of the dam with semen from different sires can explain at most 20% of the incidents of rejected paternity.

The number of cows genotyped, the number of cows with rejected paternity, and the frequency of rejected paternity and the model III least square means of the 5 inseminators with the highest proportion of rejected paternity, and the 5 inseminators with the lowest proportions of rejected paternity serving at least 50 cows are given in Table 9. The proportion of rejected paternity ranged from 27 to 82% for the 5 inseminators with the highest proportion of rejected paternity. These 5 inseminators were responsible for 12% of all the cows with identified inseminator, but for 33% of the 646 cows with identified inseminator and rejected paternity. There was one additional inseminator with a rejection frequency of >20%. Deleting the cows produced by these 6 inseminators, the overall proportion of cows with rejected paternity and identified inseminator was reduced to 8.3%. Inseminator 202, with 82% rejected paternity

Table 8. The number of cows genotyped, the number of cows with rejected paternity, and the frequency of rejected paternity by computed gestation length.

Gestation length (d)	Number of cows		Frequency of rejected paternity
	Genotyped	Rejected paternity	
240–249	6	1	0.167
250–259	13	3	0.231
260–269	350	51	0.146
270–279	3594	398	0.111
280–289	1532	183	0.119
290–300	32	10	0.312
Total	5527	646	0.117

Table 9. The number of cows genotyped, the number of cows with rejected paternity, and the frequency of rejected paternity for the 5 inseminators with the highest fraction of rejected paternity, and the 5 inseminators with the lowest fractions of rejected paternity and responsible for at least 50 cows.

Inseminator	Number of cows		Frequency of means	Least square means ¹
	Genotyped	Rejected paternity		
202	57	47	0.825	0.800
125	36	11	0.306	0.305
225	177	53	0.300	0.265
209	342	94	0.275	0.256
142	52	14	0.269	0.255
113	266	12	0.045	0.069
137	145	6	0.041	0.065
146	199	7	0.035	0.050
116	78	2	0.026	0.030
143	80	2	0.025	0.015

¹By Model III.

worked in 3 of the 5 herds with the highest frequency of rejected paternity, including herd 435000. The proportion of rejected paternity ranged from 2.5 to 4.5% for the 5 inseminators with the lowest proportion of rejected paternity and at least 50 recorded cows. The least square means were very similar to the simple means, indicating the dominance of this effect in the model III analysis. Standard errors ranged from 0.025 to 0.050. There were 2 inseminators with no cases of rejected paternity, but these inseminators were listed for only 10 and 15 cows.

DISCUSSION

Nearly all previous studies that have estimated the frequency of incorrect paternity determination have used up to 11 markers. In the current study, which was a by-product of a genome scan for QTL, 104 markers were considered. Of these, 17 were rejected because of high genotyping error rates. This is not surprising considering that in this study, markers were selected based on genomic location and polymorphism. Ease and reliability of genotyping were only secondary considerations. The 11.7% frequency of rejected paternity determinations in this study is in agreement with most previous studies on this and other populations. Ron et al. (1996) obtained a paternity rejection rate of 5.2% in this same population, based on genotyping 12 microsatellite markers with radioactive labeling on 173 cows and their 4 putative sires, but most of the cows had valid genotypes for only a fraction of the markers. Mean exclusion probability was 0.85; thus, the predicted sire misidentification rate was 6.1%. More recently, Ron et al. (2003) found a paternity misidentification rate of 6.25% for this population based on 244 elite cows. However, they

required 2 markers in conflict, and cows were genotyped for, at most, 9 markers. Single discrepancies were considered genotyping mistakes. Thus, this rate is probably an underestimate of the true value. Visscher et al. (2002) found an overall paternity error rate of 10% in the UK dairy population based on analysis of 568 cows for 11 unlinked microsatellites. Using blood groups and protein polymorphisms, Beechiner and Kelly (1987) found a paternity rate of 8 to 20% in Ireland, Christensen et al. (1982) reported rates between 5 and 15% in Danish dairy cattle, and Geldermann et al. (1986) found a misidentification rate of 13% in German dairy cattle. Finally, Bovenhuis and van Arendonk (1991) extrapolated an error rate of 12% in the Netherlands based on milk protein genotypes.

Of the 7 causes listed by Christensen et al. (1982) for paternity mistakes, the main factors appear to be 2 and 4: "AI technicians incorrectly identifying semen samples" and "errors when the bull's herdbook number or name is entered into the insemination record." As noted previously, factors 5 and 6 are not applicable under the Israeli Holstein recording system. The analysis of the insemination records indicate that factor 3, "insemination of cows already pregnant by a previous insemination," can explain at most 20% of the cases of rejected paternity. Factor 7, "interchange of calves at the farm," does not seem to be a major factor, as demonstrated by the small difference in paternity error rates between cows born on dates with multiple births as compared with cows born on dates with single births in the herd. Furthermore, the herd effect was not significant in analysis models that also included the inseminator effect.

It is more difficult to quantify the effect of the first factor: "mistakes by AI institutes in labeling semen." The effect of putative sire was significant in all models tested, and this seems to be due mostly to the contribution of sire 3099 with a rejection frequency of 22% and a least squares mean of 0.18. The most likely explanation would be a mistake in labeling a semen ejaculation from this sire at the AI institute, although there is no way to confirm this at present. Each ejaculation produces about 400 semen doses, which should result in approximately 40 milking cows. Paternity was rejected for 61 cows listed as daughters of sire 3099. This is about 30 cows more than expected, as compared with the population mean. Thus, the additional 30 cows with rejected paternity can easily be explained by erroneously attributing a single ejaculation from some other sire to 3099. It should be noted that since relatively few daughters of this sire were still alive when collection of semen began, special efforts were made to obtain every available cow recorded as progeny of this sire.

The adoption of hand-held terminals for data entry by the inseminators in Israel in the south of the country and development of a bar code system for each cow during 1993 to 1994 should have resulted in a significant reduction in paternity recording errors. Nevertheless, the linear regression of birth date in model II at $-0.005\%/yr$ was not significant. The highest year least square mean was for 1994, in the middle of the time period analyzed.

It is necessary to add an eighth factor to the seven causes for paternity rejection listed by Christensen et al. (1982): mistakes in the paternity confirmation laboratory that result in rejection of paternity of cows with correct paternity identification. Incorrect rejection of paternity by the laboratory could be caused either by genotyping mistakes, null alleles, mutation, or sample switching. As noted, the frequency of mutation for microsatellites is very low and should not be a significant factor relative to the other causes of error. Genotyping mistakes can make a major contribution to paternity rejection, especially for markers that are difficult to genotype.

Null alleles mimic genotyping errors and the extent of the null allele phenomenon may vary with different annealing temperatures used for PCR (Petersen and Bendixen, 2000). Averaged over all markers, the mean difference between observed and expected frequency of heterozygotes was virtually zero. However, there were 11 markers with an absolute difference >0.1 . Even with 300 genotypes, a difference of this magnitude is highly significant by a binomial test. Of these, 4 were markers with $>1\%$ single discrepancies, including 2 of the markers with null alleles. The reason for the large difference between observed and expected heterozygote frequency for the remaining 7 markers is not clear. Five of these markers had lower than expected frequency of heterozygotes, and may therefore also be due to null alleles, even though sires with null alleles were not detected. Furthermore, Hardy-Weinberg equilibrium assumes random mating, which clearly is not the case for this population.

We required at least 2 markers in conflict between the sire and daughter and at least 10% of the markers with valid genotypes. Assuming a 1% rate of discrepancy between the genotypes of the sire and progeny due to incorrect genotyping and mutation, the probability of erroneously rejecting paternity based on the binomial distribution would be 0.005 if 11 markers are genotyped, and at least 2 markers in conflict are required for rejection of paternity.

The effect of sample switching is more difficult to evaluate. The extremely poor results obtained in herd 435000 were originally considered suspect. Therefore, a sample of cows for this herd was independently resam-

pled, and genotypes were determined on the new sample. The results were nearly identical to the original sample.

Bredbacka and Koskinen (1999) found a mean exclusion probability of 99.91% in Holstein-Friesians using the 9 basic markers of the ISAG panel. This corresponds well with the value of 99.95% for 9 markers obtained by the regression of $\log_{10}(1 - Ex_i)$ on the number of markers. Although the regression results indicate that an exclusion probability of $>90\%$ is obtained on the average with 3 microsatellites and $>99\%$ with 6 microsatellites, in practice, more markers will be required for definitive parental confirmation. If 2 markers in conflict are required to verify exclusion and exclusion probabilities are assumed equal for all markers, then based on the binomial distribution, the probability of exclusion will be: $1 - [p^J + J(1 - p)p^{J-1}]$, where p is the nonexclusion probability, and J is the number of markers. Based on the regression of the $\log_{10}(1 - Ex_i)$ on the number of markers, the mean nonexclusion probability per marker was 0.436. Using this value, at least 6 markers are required to obtain an exclusion probability of 90%, and 9 markers are required to obtain an exclusion probability of 99%. Since not all genotypes will be readable, 11 microsatellites can be considered a reasonable minimum for paternity verification, and this is the number included in the current ISAG panel. Two markers, INRA23 and TGLA53, were added to the original 9 (Bredbacka and Koskinen, 1999). Neither of these was genotyped in the current study. Although on average 26.6 markers per cow were genotyped in the current study, there were still 457 cows (7.5%) for which paternity was considered "undetermined." It should be noted though, that the criteria used for paternity determination were much stricter than for all previous studies.

The formula used to determine exclusion probabilities assumed that the markers were independently distributed, and that the actual and putative sires were unrelated. However, as can be seen in Table 1, many of the markers analyzed were genetically linked, and many widely used sires were related. The first factor should not significantly affect the exclusion probability if the true and recorded sires are not closely related. In this case, linkage phase relationships will not be maintained, unless the markers are very tightly linked. The fact that the true and recorded sire may be related will affect exclusion probabilities. For example, if the putative and true sires were half-sibs, then there is at least a 50% chance that the 2 sires will have at least one allele identical by descent for any particular marker.

Various studies have estimated the effect of pedigree mistakes on the rate of genetic gain. Geldermann et al. (1986) concluded that the loss in response should be similar to the proportion of progeny misidentified. More

recent studies have found smaller losses. Israel and Weller (2000) conducted a stochastic simulation study of a large dairy cattle population undergoing selection to investigate the effect of a 10% incorrect paternity on genetic progress over a 20-yr period. They estimated that the annual rate of response was reduced by 3 to 4%. Visscher et al. (2002) predicted a loss in response of 2% for one round of sire selection using the same heritability (0.25) and progeny group size (100), as a result of reduced reliability. This difference between these 2 studies may reflect both the impact of incorrect identification of both sexes (e.g., daughters of dams with incorrect pedigrees occurred in the simulation of Israel and Weller (2000)) and the accumulated effect of pedigree errors over multiple generations. Banos et al. (2001) found that a postulated paternity error rate of 11% for sires used in international genetic evaluations decreased genetic trends by 11 to 15%.

By genotyping approximately 1000 cows per year, it should be possible to identify problematic insemination technicians. This should reduce the rate of paternity error from approximately 12 to 8% and result in at least a 1% increase in the rate of genetic gain. Using vaginal swabs (Ron et al., 2003), the cost of obtaining DNA samples is negligible compared with genotyping cost of approximately US\$25 per individual (J. McSweeney, H. Winkeler, and C. Weimann, personal communication). Thus, the total costs for genotyping 1000 cows/yr will be about \$25,000. If the annual rate of genetic gain is 100 kg of economically corrected milk per year, then an additional 1% genetic gain will be 1 kg/yr. Assuming a profit value of \$0.1/kg and a population of 100,000, this translates into a nominal annual gain of \$10,000/yr. However, genetic gains are cumulative and eternal. Weller (1994) showed that with a discount rate of 8%, a lag of 5 yr prior to first gain, and a profit horizon of 20 yr, the break-even point between costs and returns will be reached within 20 yr if nominal annual gains are at least 0.31 of the nominal annual costs. Thus, the proposed "quality-control" program is economically justified. In addition, the DNA samples collected could be used for additional studies.

CONCLUSIONS

The overall frequency of rejected paternity was 11.7%. The main causes of incorrect paternity recording appear to be inseminator recording mistakes, and possibly mistakes with respect to semen labeling at the AI institutes. Incorrect paternity recording due to multiple inseminations by different sires could explain at most 20% of the paternity mistakes. Switching between calves born on the same day is not a major factor in paternity errors. Instituting a system of quality control,

especially at the level of the inseminator, should reduce paternity errors to no more than 8%, and increase genetic progress by at least 1%.

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