

Bioethics: A Scientist's Guide to Approaching Bioethics

26 Bioethical considerations of food animal products and production. W. R. Stricklin*, *University of Maryland, College Park*.

Humans obtain a number of products from animals. These include food, clothing, medical materials, companionship and research knowledge to name a few. A valued ethic contends that it is inappropriate for one to treat another as a means to an end. In short, it is wrong to treat another as simply a product. Historically, non-human beings have not been included as "others" and thus their treatment as products has been generally accepted. However, a growing view holds that animals are "subjects of a life" and deserving of consideration beyond their economic worth. Accordingly, the public asks increasingly for assurance of appropriate animal well-being. At the same time, polls indicate that the public majority does not wish to give up using animal products. The research community has dealt with these somewhat conflicting viewpoints by adopting animal care and use committees (ACUC). Research animals continue to provide a product (i.e., data points) for researchers, but the ACUC, not the researcher, is responsible for the animal's life, i.e., its care, treatment and well-being. This uncoupling of the research animal's life from the animal as a product via the ACUC has generally been successful and may serve somewhat as a model. But ultimately, animal agriculture faces the challenge of developing its own methodology for uncoupling the animal "as a subject of a life" from the animal product, and doing so without destroying the integrity and economic viability of animal agriculture. Third-party accreditation programs can help, but input from educators and possibly additional methods from industry leaders are also needed. Addressing the ethical implications of treating animals as subjects of a life, and not simply as products, meets with the longterm goal of animal scientists. Accordingly, developing an animal agriculture that is bioethically grounded should be consistent with developing a system that is sustainable.

Key Words: bioethics, animal products, animal welfare

27 Thinking critically about bioethical issues. K. K. Schillo*, *University of Kentucky, Lexington*.

Animal scientists embrace a humanist ideology; i.e., a perspective that emphasizes the characteristics, experiences and interests of *Homo sapiens*. According to this view, humans are so different from all other organisms that they deserve supreme status over the rest of nature. The

ability to reason in an abstract manner is the basis for this distinction. Humanists believe that this degree of intellectual ability allows humans to ascertain a true understanding of nature and that such knowledge can and should be used to advance the species. This idea underlies an ethical framework that assumes that rational analysis leads to discovery of what is right and wrong thereby perpetuating moral progress and improving the human condition. No matter how popular and appealing this approach might be it should not be immune to critical analysis. Before we begin to think critically about bioethical issues we should think critically about ethics itself. I should like to establish a framework for doing so, by discussing two major concerns. First, we should recognize that humanism is an anthropocentric perspective and may not be compatible with the overall structure and function of nature. More specifically, we should consider whether the notion of ethics is compatible with the biological principles that govern all life, including humans. Second, it is questionable that human intelligence provides the only or best means to understand or cope with nature. Basic emotions may be just as useful as or even more useful than reason in helping humans live skillfully, if not ethically.

Key Words: bioethics, humanism, rationalism

28 A pedagogical tool for scientists faced with ethical issues. C. C. Cronney*, *The Ohio State University, Columbus*.

In the United States, escalating concerns about current farm animal science and production methods have resulted not just in increased food animal protection policies, but also, animal welfare legislation. Animal scientists and industry leaders are apprehensive that such policies may primarily be driven by emotion and lack of scientific understanding, and thus, may have unforeseen consequences. The potential impacts of animal care and use decisions on producers, animals, concerned citizens, and implications for the environment and food prices must also be considered. Balancing the interests and values of all stakeholders has presented a considerable challenge. An ethics assessment process developed for addressing biomedical ethics issues presents a more inclusive model to combine socio-ethical concerns with relevant factual information, thereby facilitating decision-making that is both ethically responsible and pragmatic. A case study will illustrate application of this model, which includes identification of the ethical problems, the embedded values, the relevant facts and moral tests that can be applied.

Key Words: ethics, science, values

Breeding and Genetics: Dairy Cattle Breeding I

29 Using veterinary and milk recording data for a genetic analysis of health traits. J. Moro-Méndez*¹, E. Boucharde², and R. I. Cue¹, ¹*McGill University, Ste-Anne-de-Bellevue, QC, Canada*, ²*Université de Montréal, Faculté de Médecine Vétérinaire, Saint-Hyacinthe, QC, Canada*.

The objective was to estimate clinical incidence of diseases (CI) and the genetic variability of health traits in commercial dairy herds. Health events from a veterinary health database were obtained from Dossier Sante Animal (DS@HR); 296,170 cow-calving dates from 123,867 cows. Animal identification and pedigree information were obtained via Valacta and CDN and merged with the health event data. Merges

were performed for Holstein (HO) and Ayrshire (AY). This process produced 155,740 and 8,130 herd-cow-date of calving records from 70,168 HO and 3,365 AY cows, in common between DS@HR and Valacta/CDN files, with 197,755 and 10,797 HO and AY health event records, respectively. Then, binary traits were created for each health event-parity combination: coded 1 when the cow had the disease during the lactation, otherwise the trait was coded as 0. Binary traits were created for milk fever (MF), retained placenta (RP), cystic ovaries (CO), displaced abomasum (DA), mastitis 1st case and 2nd cases (M1, M2, respectively), reproductive (RP), digestive (DG), locomotive (LO), and metabolic (ME) problems. Lactational incidence rates (LIR) were calculated from the CI and the number of lactations at risk for each herd.

For HO, LIR for first to third parities ranged from 0.10% (MF) to 13% (M1). Using a sire model (year of calving and age at calving as fixed effects, herd and sire as random effects) heritabilities were calculated. Each analysis was performed specifying a binomial distribution for the dependant variable, and a logit link function (using the procedure Glimmix of SAS). The heritabilities showed low to moderate values (HO, first parity): 0.15 (DG), 0.27 (RP), 0.15 (CO), 0.09 mastitis (1st and 2nd cases combined), 0.05 (LO), and 0.05 (ME). Combining health and milk recording data is not trivial and overall correct permanent identification and recording of date of calving are critical.

Key Words: health traits, incidence, heritability

30 Use of linear and threshold models for analysis of producer-recorded health data in Holstein cattle. T. F.-O. Neuenschwander¹, F. Miglior^{*2,3}, J. Jamrozik¹, and L. R. Schaeffer¹, ¹*CGIL, Dept. of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada*, ²*Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada*, ³*Canadian Dairy Network, Guelph, ON, Canada*.

Eight health traits (mastitis, lameness, cystic ovarian disease, left displaced abomasum, ketosis, metritis, milk fever and retained placenta) are recorded by dairy producers in Canada since April 2007. The objectives of this study were 1) to estimate variance components for 8 diseases recorded in Canadian Holsteins using univariate or multivariate analyses, 2) to compare threshold and linear models and 3) to estimate the effect of days at risk on the estimates of variance components. Binary traits (0=sick, 1=healthy) were analyzed either individually or grouped according to biological similarities. Minimum number of disease recordings per herd was applied to ensure a sufficient quality of disease recording in the herds included in the analysis. Eight different models were implemented for each trait; 4 of them were sire linear models and the other 4 sire threshold models. The differences among the 4 linear or threshold models were the inclusion of days at risk and/or permanent environmental effect in addition to herd, parity and sire effects. Data included 46,104 cases of any of the above diseases. Incidences ranged from 2.6% for ketosis and 9.7% for mastitis. Metritis and milk fever had an incidence below 4.0%. Heritability for all traits with all models was below 0.04 with a linear model. The highest heritability was for left displaced abomasum (0.034). Heritabilities for lameness and metritis were below 0.01. Heritabilities on the liability scale calculated with a threshold models were between 0.003 (metritis) and 0.216 (left displaced abomasum). Converted to the observable scale, these values were close to those estimated with a linear model. There was a moderate positive genetic correlation between left displaced abomasum and ketosis as well as between metritis and retained placenta. The effect of days at risk was not significant and showed a negative sign for some traits. This may be due to the short period at risk of these traits and the highest risk of culling of sick animals.

Key Words: variance components, health traits, threshold models

31 Comparison of service-sire fertility evaluations formerly or currently available to the US dairy industry. H. D. Norman*, J. L. Hutchison, and J. R. Wright. *Animal Improvement Programs Laboratory, ARS, USDA, Beltsville, MD*.

A new service-sire fertility evaluation, Sire Conception Rate (SCR), was initiated in August 2008 to replace the previous evaluation, Estimated Relative Conception Rate (ERCR), used by the dairy industry for 20 yr.

This study derived and compared evaluations from these 2 procedures using both an August 2008 and January 2009 data cutoff (using data from the same herds) with the Western Bull Fertility Analysis (WBFA). SCR and ERCR inputs were from 3 dairy records processing centers; WBFA data was from 1. The SCR model includes 10 effects to help predict bulls' conception rate (CR). Minimum inseminations/bull in the last 4 yr to publish SCR was 300 for Holsteins (HO), 100 or 200 for other breeds. ERCR uses only first services, and WBFA and SCR use inseminations through fifth and seventh service, respectively. Comparing the January 2009 evaluations, the most bulls were summarized with SCR; number of HO bulls with SCR, ERCR, and WBFA were 2358, 956, and 1594; number of Jersey (JE) bulls were 210, 82, and 98. Mean of bull evaluations from all 3 methods were near zero. Standard deviations (SD) for HO and JE for SCR were 2.42 and 2.51, 1.76 and 1.88 for ERCR, and 2.02 and 2.72 for WBFA. Holsteins bulls that were <3, 3 to 4, 4 to 5, 5 to 6, and >6 yr had mean SCR of -0.76, -0.12, 2.30, 1.48, and 1.31%, respectively. Comparable means for ERCR were 0.89, 0.10, 0.82, 0.06 and 0.02, and for WBFA were -0.14, -0.01, -0.13, 0.38, and 0.53. Standard deviations for SCR for these same were 2.20, 2.47, 2.29, 2.22, and 2.05. Standard deviations for ERCR were 1.68, 1.79, 1.74, 1.74, and 1.98, and for WBFA were 1.85, 1.96, 2.19, 2.10, and 1.93. Correlations between SCR and ERCR for HO and JE were 0.73 and 0.76. Correlations between SCR and WBFA were 0.65 and 0.68, and between ERCR and WBFA were 0.63 and 0.73. Correlations between August 2008 and January 2009 SCR were 0.95 and 0.94. Correlations between August 2008 and January 2009 ERCR were 0.91 and 0.90, and between the same for WBFA were 0.97 and 0.96.

Key Words: bull fertility, conception rate, evaluation

32 Analysis of accounting for production in the genetic evaluation of direct herd life in Canadian Holsteins. A. Sewalem^{*1,2}, G. Kistemaker², and F. Miglior^{1,2}, ¹*Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada*, ²*Canadian Dairy Network, Guelph, ON, Canada*.

In Canada, the genetic evaluation for direct herd life (DHL) is based on cows' survival from 1st calving to 120 DIM, from 120 DIM to 240 DIM, from 240 DIM to 2nd calving, survival from 2nd to 3rd calving, and survival from 3rd to 4th calving. The multiple-trait animal model includes the fixed effect of herd year, fixed effect of registry status x herd size change x season of calving (RHS), linear and quadratic regression of age at first calving, protein yield by RHS, protein and fat yields linear, quadratic, and cubic regressions and the random effect of animal and residual (Model 1). Two additional models were developed: a) same as Model 1 without production traits (Model 2) and b) same as Model 1 except that linear and quadratic regression of protein and fat yields were fitted within quota year (Model 3). The three models were compared in terms of: a) correlation analyses of DHL bull proofs with production traits; b) predictability of DHL bull proofs; and c) Genetic trends. The same genetic parameters were used for all the three models. The results showed that the correlations between direct herd life and production traits were negative in Model 1 and positive in Model 2. However, Model 3 has resulted in zero correlations with the main production traits. There were no major noticeable differences among three models in terms of predictability and genetic trends. Therefore, modifying the current genetic evaluation model system, by fitting production traits as a regression within quota year will result a desirable correlation with production traits and will better account for potential changes over time of culling for production.

Key Words: herd life, multiple-trait animal model, accounting for production

33 Estimates of residual feed intake in Holstein dairy cattle using an automated, continuous feed intake monitoring system. E. E. Connor^{*1}, J. L. Hutchison², H. D. Norman², and R. L. Baldwin, VI¹, ¹USDA-ARS, Bovine Functional Genomics Laboratory, Beltsville, MD, ²USDA-ARS, Animal Improvement Programs Laboratory, Beltsville, MD.

Improving feed efficiency of cattle is a primary goal in livestock production to reduce feed costs and production impacts on the environment. In dairy cattle, studies to estimate efficiency of feed conversion to milk production based on residual feed intake (RFI) are limited primarily due to a lack of individual feed intake measurements available for lactating cows. The goal of this study was to apply state-of-the-art radio-frequency identification equipment to measure feed intake in Holstein cows during the first 90 days of lactation (DIM) and characterize the RFI trait in dairy cattle. A total of 107 animals (55 first-lactation heifers [L1]; 32 second-lactation cows [L2]; twenty ≥ 3 lactation cows [L3+]) were evaluated between Oct 2007 and Nov 2008. Animals were housed in a free-stall barn and individual daily feed consumption was monitored continuously using the GrowSafe 4000 System (GrowSafe Systems, Airdrie, Canada; 33 nodes), at a maximal density of < 2 cows per node. Cows were fed a total mixed ration 3 \times daily, milked 2 \times per day, and weighed every 10 to 14 d. Milk yield was measured at each milking. Feed %DM was measured daily and nutrient composition was analyzed from a weekly composite. Milk composition was analyzed weekly, alternating AM and PM milking periods. Estimates of RFI were determined as the difference between actual intake and predicted feed and energy intake based on the equation $b_0 + b_1 * BW^{0.75} + b_2 * gain + b_3 * energy-corrected\ milk$. A subset of 11 L1 heifers was evaluated for a 305-d lactation to determine the relationship between RFI during the first 90 DIM and the full lactation. Using this system, RFI ranged from -2.2 to 3.6 kg/d for L1, -3.0 to 2.6 kg/d for L2 and -2.9 to 3.2 kg/d for L3+, meaning a difference of 6.6 kg/d in DMI between the most and least efficient animals across all lactational groups. Regression analysis indicated RFI of L1 during the first 90 DIM was predictive of RFI during the full lactation ($r^2 = 0.48$; $P = 0.02$). Based on our small sample size, heritability of the RFI trait was estimated to be very low ($h^2 = 0.05$).

Key Words: dairy cattle, feed efficiency, residual feed intake

34 Trends for monthly changes in days open in Holsteins. M. Pszczola^{*1,2}, I. Aguilar^{1,3}, and I. Misztal¹, ¹University of Georgia, Athens, ²Animal Breeding and Genetics Group, Wageningen University, Wageningen, the Netherlands, ³Instituto Nacional de Investigación Agropecuaria, Las Brujas, Uruguay.

A reaction norm approach was used to estimate trends for days open (DO) with a model that indirectly accounted for heat stress. Data included 3.4 million first-parity records of DO of US Holsteins. A fixed effect model included herd-year, month of calving within region (MOC), age class, and regression on 305-d milk yield. An index calculated from the standardized solutions to MOC derived from the fixed effect model was treated as a proxy for an index on heat stress (SI). The lowest index for any region was set to zero. The highest index was one for the Southeast, 0.56 for the Northeast, 0.54 for the Midwest, 0.33 for the Northwest, and 0.42 for the Southwest. In all regions except the Northwest, the highest DO and the corresponding highest indices were in March-April. Compared to the fixed model, the reaction norm model also included the effect of an animal and a random regression on the SI; the two animal solutions are subsequently referred to as an intercept and a slope. Genetic trends were calculated for all animals and separately for sires. For all the animals, the trend for the intercept was -0.1 d/year,

while the trend for the slope was 1 d/year. For sires, the same trends were -0.3 and 1.5, respectively. Official proofs were used to characterize the 100 top and the 100 bottom bulls with at least 50 daughters for the intercept and the slope. Compared to the top bulls, the bottom bulls for the intercept gave 56 kg more milk, and their type performance index (TPI) was higher by 212 points. For the slope, the same numbers were -435 kg and -242 pt., respectively. Trends for seasonal changes of days open are unfavorable.

Key Words: dairy cattle, fertility, heat stress

35 Effects of milk fat composition, DGAT1 and SCD1 on fertility traits in Dutch Holstein cattle. R. M. Demeter^{*1,2}, G. C. B. Schopen¹, A. G. J. M. Oude Lansink², M. P. M. Meuwissen², and J. A. M. van Arendonk¹, ¹Animal Breeding and Genetics Centre, Wageningen University, Wageningen, the Netherlands, ²Business Economics Group, Wageningen University, Wageningen, the Netherlands.

Recently, selective breeding was proposed as a means of changing the fat composition of milk to improve its nutritional quality. Before implementing such breeding strategies, effects on other economically important traits should be investigated. The objective of this study was to examine the effect of milkfat composition, DGAT1 gene, and SCD1 gene on fertility traits in commercial Holstein Friesian dairy cattle in the Netherlands. Morning milk samples from 1,745 first-lactation Holstein Friesian cows were collected. From the records on reproductive performance, eight fertility traits were derived. Linear mixed models were fitted as randomized block designs, with herds as random blocks and individual cows as units of analysis. The model included days in milk, age at first calving, season of calving, and sire type. Relationships between individuals were accounted for. We found that greater concentrations of trans fatty acids (TFAs) and conjugated linoleic acid (CLA) within total milkfat had a negative effect on fertility. Increased proportions of TFAs are associated with longer intervals between calving and first insemination, larger numbers of inseminations, lower calving rates, lower nonreturn rates for insemination after first service, and lower calving rates after first service. Greater proportions of CLA were associated with longer periods to first insemination and lower nonreturn rates for insemination 90d after first service. DGAT1 affected nonreturn rates for insemination 28d and 56d after first service. Results for DGAT1 displayed a nonlinear pattern, with heterozygous genotypes associated with lower estimates than homozygous genotypes. Results suggest that breeding to decrease TFA concentrations could improve fertility, whereas breeding to increase CLA content might impair some of the reproductive traits. Regarding DGAT1, results suggest that selecting for animals with the AA genotype would be the most desirable with regard to the nutritional quality of milk and the reproductive performance of cows. Results of this study can be used to assess the economic effects of breeding to change milk fat composition on reproduction.

Key Words: dairy cattle, milk fat composition, fertility

36 Deriving final score from linear traits for the Italian Holstein cattle. S. Biffani, F. Canavesi^{*}, and R. Finocchiaro, ANAFI, Cremona, Italy.

Over the past ten years the definition of Final Score has been changed quite a few times due to the harmonisation of type traits evaluation. Moreover, the increasing importance of cow functionality, with particular emphasis on feet & legs, has not been completely taken into account.

All these changes can affect the robustness of genetic evaluation since the definition of the trait is not the same across time. To address this issue, and to guarantee a better comparison of bull and cow EBVs across time and between national and foreign bulls, a formula to derive final score from linear conformation traits EBVs has been developed using the selection index theory. At first a new phenotypic definition of Final Score was defined combining 15 different linear traits according to the last recommendations of the international harmonisation group. Subsequently genetic correlations were estimated between the new definition of Final Score and each of the 15 linear traits scored in Italy. Selection index theory was then applied to maximize genetic gain for Final Score making use of the correlated traits. A total of 15 linear traits were used to estimate Final Score resulting in 31% contribution linked to frame and dairy strength traits, 21% to feet & legs and 48% to udder traits. The new Final Score will be introduced officially in Italy during 2009.

Key Words: EBV, final score, linear conformation traits

37 Modelling technical parameters of individual extended lactation curves in Italian Holsteins. R. Steri¹, E. L. Nicolazzi², G. Gaspa¹, F. Canavesi², C. Dimauro¹, and N. P. P. Macciotta*¹, ¹*Dipartimento di Scienze Zootecniche, Università di Sassari, Sassari, Italia*, ²*Associazione Nazionale Allevatori Frisone Italiana, Cremona, Italia*.

In several countries, the average length of lactation is increasing due both to reproductive failure but also to management strategies. The investigation of basic traits of individual extended lactations may be of

interest for selection programmes. In this study, 68,899 individual lactations of 45,132 Italian Holstein cows were fitted with two mathematical functions that have been previously tested on extended lactations: the Ali and Schaeffer multiple regression function (AS) and the non linear modification of the Dijkstra model (DJ). Lactations were of three parities (first, second and third) and were classified into four lactation length classes (1<350 d; 2= from 351 to 450d; 3= from 451 to 650d; 4>651 to 1000d). Both models were fitted to individual lactation curves for milk yield (MY), fat (FP) and protein (PP) percentage and somatic cell score (SCS). Peak yield (Y_m), time of peak yield (T_m), time of inflection point (T_f) and final test day production (for AS) or asymptotic production (for DJ) were calculated. AS and DJ function show a good fit when modeling lactations curves of milk yield and protein content in 651-1000d class: the percentage of curves having an R-square higher than 0.7 was about 75% for MY, 60% for PP, 15% for FP and 15% for SCS. Estimates of technical parameters show a remarkable variability. As an example, for milk yield the median of asymptotic level of production estimated with DJ was kg -7.3, kg 11.5 and kg 8.51 for first, second and third parity respectively, whereas the production at last test estimated by AS function was kg 5.5 kg, kg 8.4 and kg 5.5 for the same parities. Time of inflection (T_f) also show a great variability between models: for first parity cows; for example, it was estimated as 137.7 d by DJ and 596 by AS. Finally, time at peak occurrence (T_m) was at days 82.5, 53.6 and 52.9 for DJ and 49.5, 33.7 and 41 days for AS. Both models were able to highlight some basic traits of the curve shape for individual extended lactations, although a great variability has been also detected.

Key Words: extended lactations, individual curves, mathematical modelling

Breeding and Genetics: Molecular Genetics I

38 Hybridization quality diagnostics using control probes on long-oligonucleotide microarrays: An application to the Pigoligoarray. J. P. Steibel*¹, M. Wysocki², V. D. Rilmington¹, A. M. Ramos^{1,3}, J. K. Lunney², and C. W. Ernst¹, ¹*Michigan State University, East Lansing, Michigan, BARC, ARS, USDA, Beltsville, MD*, ²*Wageningen University, Wageningen, the Netherlands*.

Long oligonucleotide microarrays are composed of 40- to 70-mer oligonucleotides spotted on glass slides. This type of expression profiling platform has been recently made available to several livestock species. Such microarrays commonly include negative control oligonucleotides (scrambled sequences) and perfect match/mismatch oligonucleotide sets (PM/MM). Graphical diagnostics (e.g. MA plots) are commonly used in exploratory microarray analysis, but they do not explicitly consider control oligonucleotides. The objective of this work was to develop graphical diagnostics that use control oligos to evaluate hybridization specificity. We re-analyzed data from 8 previously conducted experiments using the Pigoligoarray. A total of 160 microarray slides were processed under a range of stringency and hybridization conditions that resulted in various degrees of non-specific hybridization. The arrays were classified as "good" or "poor" hybridization quality depending on the extent of cross-hybridization. Negative control oligos had median signal intensities similar to or lower than non-control oligos for the "good" arrays whereas for the "poor" arrays negatives exhibited an almost symmetric distribution of intensities around the median for non-control oligos. The analysis of PM/MM sets was even more informative, clearly indicating whole experiments or arrays with extensive non-specific binding. In experiments with specific hybridization, individual probe analyses of PM/MM sets revealed the expected decrease in signal inten-

sity with increased MM number. Additionally, the dispersion around the median intensity decreased as the number of mismatches decreased. Such patterns were not present on arrays with nonspecific hybridization. While these diagnostics were developed using the Pigoligoarray, the proposed applications could be straightforwardly implemented for other long-oligonucleotide microarrays that include the same types of control probes, such as for the bovine and turkey. In summary, we successfully used PM/MM and negative oligos to elaborate graphical diagnostics of hybridization quality.

Key Words: microarrays, cross-hybridization, graphical diagnostics

39 Low density SNP chip for non-genotyped animals. H. Wang*¹ and R. Rekaya^{1,2}, ¹*Department of Animal and Dairy Science*, ²*University of Georgia, Athens*.

Availability of reliable and inexpensive genotyping platforms for single nucleotide polymorphism markers (SNP) has made the possibility for genomic breeding value estimation in several livestock species a reality. Unfortunately, at above two hundred dollars per animal this technology is still too expensive for massive use at the commercial level. Currently, this technology is mainly used for genotyping top animals and then used in two step procedures for estimating genomic breeding values. For its use at the population level, the SNPs genotypes of non-typed animals have to be inferred somehow from the already genotyped animals and their relationships. In fact, several attempts have been proposed ranging from the calculation of gene content to the construction of a covariance matrix similar to the classical additive relationship matrix.