GENETIC EVALUATION CARDS UPDATED APRIL 2019

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1. THE SELECTION INDEX AND OTHER COMPOSITE INDEXES

1.1. PFT (Productivity, Functionality and Type)

PFT is the selection index of the Italian Holstein breed, which combines milk production, functionality and type. The official ranking is based on PFT and ranges from 0 to 99 dividing the population in percentiles. The ranking is calculated separately for bulls and cows. The rank 99 identifies the best 1% of bulls and cows. Since December 2011, rank 98 (best 2% of the population) is the official limit for a bull to be used in A.I. The following table shows the relative weight of each trait included in PFT.

The formula for PFT is as follows:

```
PFT = 12.50 * (0.32 * fat kg + 1.79 * protein kg + 0.087 * fat% * 100 + 0.28 * protein% * 100 + 4.04 * type + 13.93 * ICM + 6.07 * IAP + 9.21 * ((longevity-100)/5) + 11.51 * ((somatic cell score-100)/5,70)) + 10.59 * ((fertility-100)/5))
```

which determines a 49:28:23 ratio between production, conformation and functionality.

PRODUCTION	WEIGHT	FUNCTIONALITY	WEIGHT	CONFORMATION	WEIGHT
Milk kg	0	Longevity	8	Type	4
Fat kg	8	Somatic Cells	10	Udder (ICM)	13
Protein kg	36	Fertility	10	Feet & Legs (IAP)	6
Fat %	2				
Protein %	3				

1.2. ICM (Udder Composite Index)

The goal of the ICM index is to breed for a functional udder. Its formula is based on percentages of weights for the breeding values based on the linear scores of the udder, defined according to their relation with the functional longevity of the animals and is as follows:

ICM = 0.19 * fore attachment strength +

0.17 * rear attachment height +

0.21 * udder support +

0.26 * udder depth +

0.17 * front teat placement

1.3. IAP (Feet & Legs Composite Index)

The IAP index, used for the first time in May 2000, combines three indices based on linear traits relative to feet and legs and locomotion evaluated by the classifier. The weights express (as a percentage) the relative importance of each trait (due to correlations) in relation with the trait 'feet & legs functionality', and are the following:

```
IAP = 0.16 * foot angle +
0.244 * rear leg rear view +
0.408 * locomotion +
-0.208 * abs(rear legs side view + 1)
```

1.4.TYPE

From December 2014, the index for TYPE is derived from 17 indices based on linear traits to ensure the same definition across all Italian and foreign bulls. The formula has been derived from genetic correlations among the 17 different traits and the genetic correlations with the actual definition of Final Score officially used by classifiers. Rear legs side view and rear teat placement have an intermediate optimum. Type is calculated as follows:

```
TYPE = 0.000 * stature + 0.128 * strength + 0.031 * body depth + 0.158 * angularity + 0.044 * rump angle + 0.013 * rump width - 0.025 * abs(rear legs side view + 1) + 0.038 * rear legs rear view + 0.050 * foot angle + 0.175 * udder front + 0.072 * rear height + 0.030 * udder support + 0.014 * udder depth + 0.050 * front teat placement + 0.023 * teat size - 0.032 * abs(rear teat placement + 1) + 0.016 * locomotion
```

2. ECONOMIC - FUNCTIONAL INDEX (IES)

2.1. IES (Economic and Functional)

IES is a selection index which has the aim to maximize the genetic progress, both in the economic sense as well as for health and welfare traits. The index is based on an estimate of average costs and revenues in the entire cow productive life. Revenues considered are the average milk cost and quality parameters payments. Costs are quantified taking into account costs for rearing heifers, the dry matter intake needed for growth, maintenance and production, and it has to consider also, information on health and welfare derived from genetic and genomic indexes (fertility, mastitis resistance and calving ease). This index is expressed as economic difference of any single individual (or the bull's daughters) with respect to the genetic base. The index will be updated periodically, either for the economic value estimation, in order to use updated market prices, either for the introduction of new genetic indexes that will be set up such as animal welfare, mastitis resistance, feed efficiency or reduction of environmental impact. The index is expressed as net income (revenue – cost), estimated on the entire cow productive life $(\mathbf{\epsilon})$, with respect to the average cow population. This selection index is published for AI bulls and for genotyped females. The following table shows the relative weight of each trait included in IES. The formula for IES is as follows:

```
IES = 0.32 * fat kg + 1.37 * protein kg + 0.043 * fat% * 100 + 0.0188 * protein * 100 - 5 * stature + 4 * locomotion + 1 * udder depth + 20.51 * (longevity – 100)/5 + 6 * ((scs-100)/5.7) + 18 * (fertility-100)/5) + 3.51 * ((maternal calving ease -100)/5)
```

which determines a 39:51:10 ratio between production, functionality and conformation.

PRODUCTION	WEIGHT	FUNCTIONALITY	WEIGHT	CONFORMATION	WEIGHT
Milk kg	0	Longevity	20.51	Stature	-5
Fat kg	8	Somatic Cells	6	Locomotion	4
Protein kg	27.62	Maternal Calving Ease	3.51	Udder Depth	1
Fat %	1	Fertility	18		
Protein %	2	Body Condition Score	3.35		

3. CHEESEMAKING AND SUSTAINABILITY INDEX – PARMIGIANO REGGIANO

3.1. ICS-PR (Cheesemaking and Sustainability Index – Parmigiano Reggiano)

ICS-PR is a selection index which has the aim to select those animals whose daughters maximize the profit for farms whose milk produced is intended for long-ripening cheese production. This index has to take into consideration the new breeding regulations and it has to be profitable for farmers, furthermore it has to be sustainable in the ethic, economic-social and environmental contexts. The index is based on an estimate of average costs and revenues for each lactation in the entire cow productive life. Costs are quantified taking into account costs for rearing heifers, feed costs considering the dry matter intake needed for growth, maintenance and production, costs for cheese-making and transport, and it has to consider also, information on health and welfare derived from genetic and genomic indexes (fertility, mastitis resistance and calving ease); revenues considered are those estimated for the entire cow productive life and the cow's value at culling. For cheese yield prediction, expressed as kg of cheese, fat and protein percentage, somatic cells count (SCC) and k-casein genotype are considered. The index is expressed as net income (revenue - cost), estimated on the entire cow productive life $(\mathbf{\xi})$, with respect to the average cow population. Bulls used to derive index weights had a reliability of at least 50% and rank greater than 59. The following table depicts the relative weight of each trait included in ICS-PR. The formula for ICS-PR is as follows:

```
ICS-PR = 0.05 * fat kg + 0.30 * protein kg – 0.02 * stature + 0.03 * locomotion + 0.02 * udder depth + 0.14 * ((scs-100)/5.70) + 0.09 * ((maternal calving ease-100)/5) + 0.10 * ((longevity-100)/5) + 0.05 * ((mastitis-100)/5) + 0.20 * ((fertility-100)/5)
```

To this formula needs to be added a premium:

- 0.10 € / day of life for k-casein BB bull's daughters
- 0.05 € / day of life for k-casein AB bull's daughters

which determines a 35:58:7 ratio between production, functionality and conformation.

PRODUCTION	WEIGHT	FUNCTIONALITY	WEIGHT	CONFORMATION	WEIGHT
Milk kg	0	Longevity	10	Stature	-2
Fat kg	5	Somatic Cells	14	Locomotion	3
Protein kg	30	Maternal Calving Ease	9	Udder Depth	2
		Fertility	20		
		Mastitis	5		

4. BREEDING VALUES FOR PRODUCTION TRAITS AND SOMATIC CELL SCORE (SCS)

4.1. Data recording

ANAFIJ receives every week from the Italian Breeders Association (AIA) all the milk records collected by the personnel of the Regional Breeders Associations (ARAs). Alternate recording data (AT) are projected to 24 hours records complying ICAR standards. All available records are used. Every lactation has a weighting factor expressing its accuracy:

• AT records are worth 98% of monthly complete records (A records).

All the records collected between day 5 and day 305 of a lactation are used, up to at most three lactations. Besides ordinary data editing, records abnormally deviating from each cow's estimated lactation curve are discarded.

4.2. Environmental effects

A random regression test day model is adopted, estimating every effect using a function that explains their variability from day 5 to day 305 of lactation. Breeding values for milk, fat, protein and somatic cells are estimated simultaneously, thus making use of genetic correlations among traits that improve total reliability of estimates. Estimated fixed effects are age at calving, calving season (spring or autumn), area of production (Northern, Central or Southern Italy and "Parmigiano Reggiano cheese area") and the five-years period in which production occurred (from 1990).

Average variability of milk, fat and protein within herds are calculated every year, using all available test-day records; this parameter measures variability of herd productions around total average and helps in identifying higher and lower than average variability herds before adjusting them to match the "correct" scale. The "correct" scale is within-herd variability of the genetic base cows. This correction for heterogeneity of variance allows differences among animals to be measured in a common scale for all herds. Animals are compared according to herd-year-test day and parity. Cows are divided into a first and a multiple parity class; this last effect allows to account for every management effect within herd and time.

4.3. Random effects

The <u>animal</u> effect, after which the Animal Model statistical method is named, allows for the estimation of breeding values of all animals within the population. In the random regression test day model the hypothesis is that animals express a different genetic value each day of lactation in response to environmental stimulation. A function is therefore estimated for each animal based on five parameters. Besides, all animals with (cows) or without (bulls) production records are considered simultaneously, together with all their relationships, allowing for the deviation from contemporaries

to be resolved into its dam and sire components and taking into account, for instance, the fact that on the best cows usually the best available bulls are used. A second random effect is the <u>permanent environmental effect</u>: each cow can have more than one lactation. Therefore, it is necessary to estimate occasional management effects that may have a negative (a mastitis which severely damages one of the quarters), or a positive (a very positive interaction with an healthy environment) influence on her lifetime production. Also in this case, a five parameters function is estimated.

4.4. How the breeding value is expressed

The traits for which breeding values are calculated are milk, fat and protein yields and somatic cell score (SCS).

4.4.1. Production traits

Heritability varies according to day of lactation and parity and usually is around 0.30, thus establishing a 30:70 ratio between genetic and environmental variability.

The three resulting breeding values, for first, second and third lactation, are then combined in an index of total three-lactation production:

```
EBV305tot = 0.333 * EBV3051 + 0.333 * EBV3052 + 0.333 * EBV3053
```

This index is expressed in kilograms as deviation from a reference base, the genetic base, that sets the zero of breeding values. The genetic base is a rolling base and it is updated in April every year; its purpose is to express the breeding values relative to the value of the reference cows. Current genetic base (2019) reflects genetic value of the cows born between 2011 and 2013. Every year the triennium will be moved forward one year.

Percentages are computed with reference to the 305 days phenotypic productions of cows of the genetic base. The base values are published on the web page in the section related to genetic proofs (http://www.anafi.it/). The base values are then used in the following formula to calculate the breeding values for percentages:

```
fat% = 100 * (fat base + fat) / (milk base + milk) – fat% base
protein% = 100 * (protein base + protein) / (milk + base) – protein% base
```

A bull is officially proven when he reaches a minimum reliability of 80% and has at least 30 daughters with 120 DIM. For all foreign bulls the results provided by INTERBULL are used (decision n° 256 of the Central Technical Committee 25/10/2013).

4.4.2. Somatic cell score

Heritability varies according to parity and day of lactation and usually ranges from 0.17 in first lactation to 0.25 in third lactation. The resulting three breeding values for first, second and third lactation are combined in a total index for SCS, that express the mean somatic cell score in the three lactations, using the following formula:

This index is expressed on a scale with mean equal to 100 and standard deviation equal to 5. Current genetic base represents the genetic value of cows born between 2011 and 2013 as for the production traits. Also, for SCS indexes, for all foreign bulls the results provided by INTERBULL are used.

4.4.3. Persistency index and maturity rate

The individual lactation curves that the random regression test-day model provide for each cow, allow us to quantify the genetic component for persistency of production; to express it with an indicator, we calculate the percent ratio between 280 days and 60 days productions. The index is on a scale with mean 100 and SD equal to 5. There are three indexes for persistency, one for each lactation, and a combined indicator computed using the following formula:

Maturity rate measures, on the other hand, the difference between the sum of fat and protein kilograms in third and first lactation. The result is then expressed on a scale with mean 100 and SD equal to 5. It tells us which are the bulls whose daughters improve performances through lactations (values above 100), compared to those whose daughters perform the best in first lactation (values below 100).

5. UDDER HEALTH INDEX (MST)

5.1. Data recording

Data for all cows collected every month by the personnel of the Breeder's Regional Associations (ARAs) are available. Single test-day Somatic Cells Count (SCC) data of each animal were log-transformed to somatic cell score (SCS) as SCS=log2(SCC/100,000). An index was created using all the available information in the data-set. Novel traits were validated on a sample data-set with the objective of selection (i.e. clinical mastitis) and, thanks to an available data-set with effective measurements on cow's mastitis presence (or absence) within lactation, four novel traits have shown the strongest genetic correlation. These traits are: mean SCS between 5 and 150 DIM; standard deviation of SCS within lactation; severity, defined as the ratio between the number of test-days with SCC greater than 400,000 cells/mL and the total number of test-days within lactation and peak defined as the number of peaks during lactation (number of times when SCC shows a change from <100,000 to 400,000 cells/ml on three consecutive test-days). Genetic correlations among the four traits and clinical mastitis and their heritability is depicted in the following table. These traits are combined together in an aggregate selection index to identify individuals which are more resistant to clinical mastitis.

Trait	Heritability	Genetic correlation with clinical mastitis
Clinical mastitis	0.03	
SCS5-150	0.17	0.39
SD_SCS	0.05	0.44
Severity of infection (%)	0.11	0.41
Peak	0.07	0.51

5.2. Environmental effects

The environmental factors considered in the model are: <u>herd-year-season</u>, <u>age at first calving and number of test-day records within lactation</u>.

5.3. Random effects

The only random effect is the <u>animal</u> effect, considering bulls and cows at the same time, accounting for genetic level of mating and using all available pedigree information.

5.4. How the breeding value is expressed

Currently breeding values are calculated from phenotypes mesured on first-parity cows. Heritability of the aggregate udder health index is 15%. The index is expressed on a scale with mean 100 and SD of 5. Animals with indexes greater than 100 are those animals whose offspring will be more genetically resistant to mastitis.

6. BREEDING VALUES FOR TYPE TRAITS

6.1. Data recording

Information of type evaluations carried out on all first-parity cows classified one to three times a year by the classifiers is used for the calculation of the breeding values. Unlike to what happens with the production proofs, in this case age variation is not so strong and there are no repeated data. For each cow only one classification is used.

6.2. Environmental effects

Among the animals classified in a herd there are differences by age and stages of lactation which undoubtedly influence their body condition and appearance. All traits are evaluated together in a multiple trait analysis. The classifier scores what he/she sees, therefore the statistical model must take into consideration this different condition by using an <u>interaction</u> effect <u>between age and stage of lactation by a two-year period</u>. The indexes are calculated simultaneously for all linear traits.

Cows are compared among them on an equal <u>herd-year-round of classification</u>: this allows to simultaneously take into account all managerial effects of each single herd and also the year and the classifier who evaluated the animal.

6.3. Random effects

The only random effect taken into consideration is that of the <u>animal</u>. All parental relationships existing among the population are taken into consideration, and this allows to estimate the genetic value of all animals, taking into account possible preferential or corrective mating.

6.4. How the breeding value is expressed

The breeding values of all linear type traits evaluated since 1984 and final score are calculated. The table illustrates the heritability data utilized for the calculation. A zero breeding value refers to the genetic level of animals born between 2011 and 2013 (i.e. the 2019 genetic base).

All breeding values are standardized for the variability of the cows in the genetic base. In this way, all traits can be measured on a same scale (see figure), which goes from -3 to +3 both for cows and bulls. In reality, bulls and cows do not vary in the same way, and therefore bulls that have high breeding values, especially for production traits, being all highly selected and well above the cows average, tend to vary towards extremes which are higher than 3. On the table you can see the heritability values of all traits, the phenotypic average of the animals in the genetic base, and the equivalent in points (linear scale) of one standard deviation of that breeding value. The table values vary at every base change and are updated on the web page where genetic proofs are.

Genetic Evaluation

The breeding value is published when a bull has at least 10 daughters in 5 herds, in case of bulls with daughters in Italy. Conversion of breeding values to the Italian scale is used for bulls imported from abroad with daughters still not classified in Italy. For foreign bulls the official proof is the Italian one of INTERBULL.

Genetic base 2011-2013

Linear Trait	Heritability	Average Base	SD in linear points
Stature	0.40	31.80	2.50
Dairy strength	0.25	27.54	1.39
Body depth	0.30	30.93	1.59
Angularity	0.23	28.59	1.23
Rump angle	0.23	24.96	1.61
Rump width	0.22	27.06	1.54
Rear leg set	0.16	25.53	0.99
Heel depth	0.10	26.19	0.79
Fore udder attachment strength	0.20	24.96	1.54
Rear udder attachment height	0.21	27.69	1.37
Rear udder attachment width	0.24	29.46	1.09
Udder support	0.16	28.98	1.15
Udder depth	0.30	29.52	2.27
Front teat placement	0.19	25.38	1.11
Teat size	0.19	22.86	1.19
Rear leg rear view	0.06	26.37	0.68
Feet and legs functionality	0.10	23.55	0.76
Rear teat placement	0.18	30.36	1.72
Conformation	0.14	25.71	1.32
Locomotion	0.14	21.78	0.49

7. BODY CONDITION SCORE (BCS)

7.1 Data recording

Body condition score is a visual score by the classifiers of fat covering the pelvic and lumbar regions; its scoring is based on a 1 (very thin) to 5 (very fat) scale with 0.25-point increments. In particular, the fat reserves of the thurl region, the angularity of hips and pins, and the prominence of spinous parts are evaluated by classifiers, in such a way as to be able to define the appreciation of the animal's energy reserves. The index is considered in the official national evaluation since December 2013.

7.2 Environmental effects

There are differences in age, interval since calving and production levels among the animals present in the farm which undoubtedly influence the morphological-metabolic status of the animal. The classifier scores what he sees, which is why the statistical model used takes into account these differences through the interaction effect between age and the lactation stage. Animals are compared according to herd-vear-classification day: this allows to take into account simultaneously all the internal management effects for each farm and year and the classifier who carried out the evaluation.

7.3 Random effects

The only random effect taken into consideration is that of the <u>animal</u>. All parental relationships existing among the population are taken into consideration, and this allows to estimate the genetic value of all animals, taking into account possible preferential or corrective matings.

7.4 How the breeding value is expressed

The genetic index for BCS are then expressed on a scale with mean 100 and SD equal to 5, as for other functional traits. It tells us which are the bulls whose daughters (values above 100) have a better body condition. Each standard deviation (which is equal to a 5 on the scale of the index) is worth about 0.08 points BCS: bulls with a genetic level of less than 90 have daughters with BCS equal to 2.78, bulls with EBV between 95 and 105 have daughters with BCS of between 2.94 and 3.01 and bulls with indexes above 110 have daughters with BCS equal to 3.16 points.

Genetic bulls level	Phenotipic average of daughter
Below to 90	2.78
From 90 to 94	2.85
From 95 to 99	2.94
From 100 to 104	3.02
From 105 to 109	3.09
Above to 110	3.16

8. BREEDING VALUE FOR CALVING EASE

8.1. Data recording

During milk testing, the technicians of the Regional Breeders Association also gather all available information on as many events as possible: births, deaths, sales, breeding, calving, etc. Data on calving include the dairy farmer's evaluation of the degree of difficulty, on a five categories scale:

A = easy calving

B = help needed from one person only

C = cesarean

D = difficult calving

E = embryotomy

Each category is evaluated on a difficulty scale going from 0 to 100, where easy calving is 0 and embryotomy is 100. The sex of the newborn calf is also used to further differentiate the various degrees of difficulty. Data used for the present evaluation were collected starting from 1987. Since August 2003 we discard herd-year classes that:

- had more than 92% records coded as A;
- had more than 92% records coded as B;
- had more than 15% records coded as C and over.

8.2. Environmental effects

In the model the interaction between <u>year and month of calving</u>, the interaction between <u>province and year</u> and the interaction among the age of the dam, the sex of the newborn and the parity of the cow are taken into account.

8.3. Random effects

The effects of <u>herd-year</u>, the <u>sire</u> and the <u>maternal grandsire</u> are considered as random.

8.4. How the breeding value is expressed

There are two resulting breeding values: one is the direct effect of the bull (sire of the calf) and the second one is the maternal effect or the calving ease of the daughters of the bulls which gave birth. Both are on a scale with mean 100 and SD of 5 units. Bulls with values above 100 have a higher percentage of easy calving. Heritability is near 10%, which shows that for this trait there is a strong impact of the environmental effects in determining what is really going to happen at calving.

9. BREEDING VALUE FOR MILKABILITY

9.1. Data recording

Twice a year, the people in charge of the milk recording collect also information on the milking speed of the cows. More specifically, they ask the milkers to point out the cows which are below average for milking speed. Repeated reports on the same cow will determine her degree of slowness: the cow that is always identified as below average in milking speed at each survey, will have a score of 1; the cow surveyed three times and identified as "slow" one time only, will have a score of 0.33, and so on. Her contemporaries are her herdmates of the first survey or of the time when she was first identified as "slow", if there have been variations in time.

9.2. Environmental effects

The different <u>lactation numbers</u> of the surveyed cows are taken into account; an adjustment is also made for the <u>amount of milk</u> produced on the test day and for the herd-year-test day. By doing so it is possible: a) to avoid mistaking high yields with slow milking; b) to account for different management practices, in particular for the management of the milking parlor and of the personnel in charge.

9.3. Random effects

Another relevant factor is the effect of the <u>animal</u>, therefore in the evaluation of the genetic value of the bulls, the possible slowness of the dam of the surveyed cow is taken into account.

9.4. How the breeding value is expressed

Only the sires breeding values are published and the heritability of the trait is 6%, due to the limited accuracy of the data collected. The value is expressed as a genetic value on a scale with mean 100 and SD of 5. The value 100 corresponds to the average breeding value of the 2019 genetic base (cows born between 2011 and 2013). The average phenotypic value of cows in the base are published on the web site. The phenotypic average is generally around 4%.

10. THE BREEDING VALUE FOR FUNCTIONAL LONGEVITY

10.1. Data recording

The general lactations records give a precious information: the productive career of all the Holsteins in milk recording. It's known for how many lactations an animal has produced before being culled, at what productive levels her productions were located compared to the farm average, how often she gave birth etc.

It's possible to notice, over time, the trend of the farms: some of them are stable, some are closing and others are enlarging themselves. All these data, if properly analyzed, tell us the dynamic of the elimination of animals from the farm. Next to those animals which have already closed their career, there are those who have just started it.

10.2. Environmental effects

There are some permanent factors which do not change such as the <u>first calving age</u> of an animal, that, by the way, determines her survival capacity over time. There are time dependent factors such as the <u>age effects on the lactation stage</u>, the <u>milk yield productive level</u> within the year, divided into nine classes (four under the average, average, four above the average), the <u>fat percentage productive level</u> within the year (5 classes), the <u>protein percentage productive level</u> within the year (5 classes), the <u>yearly changes in the herd size</u>, and at the end the <u>year-season</u> effect on which external factors have an influence, such as the recoveries, the market dynamics (quota) or the pathologies that lead to extraordinary elimination patterns of the animals.

10.3. Random effects

The <u>bull</u>, <u>maternal grandsire</u> and <u>herd-year-season</u> effects are considered. The applied model, based on the survival functions, derives from medical studies and is called survival analysis; it is a sire-maternal grandsire model.

10.4. The combined index

The reliability of the survival index of the bulls, in particular for the young animals, those who have all the daughters still in production, is definitely low, a little bit higher than a pedigree index. Such an index doesn't tell us much on the real survival of his daughters. But we know that from the type evaluation of the daughters it is possible to get a partial information on their longevity. From this originates the composed index for the longevity: survival index, feet & legs and udder index are combined together taking into consideration the genetic correlation between the several traits in order to compute the final combined longevity index. In particular the correlation between udder and functional longevity is 0.48 and the one between feet & legs and

longevity is 0.14.

10.5. How the breeding value is expressed

Only the index of bulls which have at least a reliability of 50% or higher is published. The heritability of this trait is around 10%. The longevity index is expressed as a genetic value on a scale with mean 100 and SD of 5 just like for the other functional traits. Minimum and maximum values are approximately 80 and 120. The bulls that give longer-living daughters have values above 100.

10.6. What does it really mean

The functional longevity concept is not easy to explain: it is not the rough longevity the one which is possible to observe in the farm, because this one is correlated with the productive level of the animals. The concept of longevity itself is related to fertility, to the health of the animal, etc.

Besides this, between functional longevity and real longevity, and by this we mean the number of lactations that the animal is able to carry on during her career, there is a precise relation.

In order to try to quantify the real difference between animals, carrier of very positive longevity genes, and those carrier of genes with very negative effects on the longevity of their daughters, we computed a simple statistic of the data for the bulls having more than 200 daughters and born between 1980 and 1988. The differences we found are listed in the below table.

Genetic Level	Average number of daughter's lactations	Average number of eliminated daughter's lactations
Lower than 90	2.30	2.10
Around 100	2.58	2.30
Higher than 110	3.20	2.90

The expected difference is one lactation.

11. THE BREEDING VALUE FOR FERTILITY

11.1. Data recording

The data bases with inseminations, calving and pregnancy scanning are the information used for genetic evaluation for fertility. On this data several checks guarantee that only good quality records will be used for genetic evaluation (about 20% of the total records are discarded). The majority of records discarded are questionable first insemination data. Together with these data bases also linear trait and lactation data bases are used. Indeed, for a complex trait as fertility, direct and indirect traits are used all together. The direct traits considered are: heifer's age at first insemination, cow's and heifer's non-return rate at 56 days, cow's interval from calving to first insemination and cow's and heifer's interval from first to last insemination. Indirect traits are: cow's BCS and cow's mature equivalent 305 days first parity lactation. All traits are measured on first parity cows and heifers in order to evaluate daughter fertility of all bulls. The five traits measured on cows are considered simultaneously in order to fully exploit the genetic correlations that exist across these traits and to maximize the estimation precision. The same happens for the three traits measured on heifers. The two estimated values are then combined together and the aggregate breeding value for fertility is the combination of the 90% of the cow's fertility breeding value and the 10% of the breeding value estimated on heifers.

11.2. Environmental effects

Every trait is affected by different environmental effects. Thus, every trait has its own statistical model. Effects associated with cow's interval from calving to first insemination and for cow's interval from first to last insemination are:

- a) herd-year-season of calving,
- b) calving month,
- c) age at calving by year of calving;

for cow's non-return rate at 56 days effects are:

- a) herd-year-season of calving,
- b) insemination month,
- c) age at calving by year of calving;

environmental effects considered for BCS are the same used for genetic evaluation for type traits:

- a) herd-year-round of classification,
- b) age at calving by stage of lactation,
- c) year of calving;

for heifer's age at first insemination effects are:

- a) herd-year of birth,
- b) month of birth;

for heifer's interval from first to last insemination and for heifer's non-return rate at 56 days factors considered are:

- a) herd-year-season of birth,
- b) year-month of insemination.

Finally, environmental effect considered for 305 mature equivalent milk yield is simply the <u>herd-year-season of calving effect</u>.

11.3. Random effects

The <u>animal</u> effect is evaluated thus considering bulls and cows at the same time, accounting for genetic level of mating and using all available pedigree information. It is a multiple trait animal model.

11.4 The combined index

At the end of the computation step eight genetic proofs are available. The objective of selection is to improve fertility in general terms. In order to achieve this in an easy and simple way the chosen objective trait is conception rate at first service. This trait is genetically correlated with each of the eight evaluated traits and the final proofs combine all of them to maximize the improvement on conception rate at first service by placing the following emphases:

Cows:

Trait	Genetic correlation with conception rate	Relative importance in the combined proof %
Days calving-first insemination	-0.30	16
Non-Return Rate 56 d	+0.77	13
Days first-last insemination	-0.89	55
BCS	+0.28	7
305 mature equivalent milk	-0.29	9

Heifers:

Trait	Genetic correlation with conception rate	Relative importance in the combined proof
Age at first insemination	-0.06	10
Non-Return Rate 56 d	+0.67	43
Days first-last insemination	-0.73	47

11.5. How the breeding value is expressed

Only the index of those bulls which have at least a reliability of 50% or higher is published. The heritability of the traits varies from 2 to 26%. The fertility index (IAF) is expressed on a scale with mean 100 and SD of 5 just like for the other functional traits, minimum and maximum values are between 80 and approximately 120. Bulls with higher fertility have values greater than 100.

11.6. What does it really mean

In spite of the low heritability values, especially for direct traits, the genetic variability among animals is very high. In the top 5% bull population group for PFT there are individuals that have an 85 proof and others that reach 110. For cows, each standard deviation (that corresponds to a value of 5 on the proof scale) above 100 corresponds to: +5% conception rate, -11 days first to last insemination interval, -3 days calving to first insemination interval and +3% on non-return rate at 56 days; whereas for heifers it correspond to: +2% conception rate, -1.45 days first to last insemination interval, +0.1 month age at first calving and +2% on non-return rate at 56 days.

The differences we found among bulls in the populations are listed in the following table:

Cows			Daughter phe	notypic average	
Bull genetic level	IFL BCS		Calving- 1 st INS	Non return rate 56 days	Conception rate
Below 95	86.81	2.96	89.24	0.54	0.28
95 to 105	73.14	2.99	87.27	0.57	0.33
Above 105	62.05	3.03	84.72	0.60	0.38

Heifers	Daughter phenotypic average				
Bull genetic level	IFL	Age at 1 st INS	Non return rate 56 days	Conception rate	
Below 95	29.80	17.1	0.75	0.58	
95 to 105	27.32	17.2	0.77	0.60	
Above 105	25.87	17.3	0.79	0.62	

12. BULL FERTILITY (BFE)

12.1. Data recording

Data used for male fertility indicator are information available in the ANAFIJ data-base, on non-return rate on 56 days (NRR56). This information is available since 2006 for all lactating cows. On the basis of this information it was possible to calculate the percentage of the non-return rate for all service bulls. The data used take into account the inseminations of the last five years and the bulls older than 15 months. In this way we use the most useful and current information, in order to avoid alterations in the final results.

12.2 Environmental effects

The model used to calculate bulls fertility takes into account the <u>age of the bull</u> and its origin including <u>bull state</u> (genomic, in progeny test, progeny tested) and <u>Al center</u> that produces the semen, and also, the cows fertility such as, the interval between calving and conception, expressed as <u>classes of days open</u>, the <u>energy used in the production of milk</u> (ECM) and <u>parity</u>. The animals are compared with each other for the same <u>herd-year-season of insemination</u>, allowing to take into account simultaneously of all the internal reporting effects at each herd.

12.3 Random effects

The bull and the <u>permanent environmental effect of the cow</u> are considered as random effects.

12.4 How it is expressed

The bull fertility indicator is expressed on scale 100 and standard deviation of 5 just like for the other functional traits. Index greater than 100 identify the more and less fertile bulls in comparison with the average population. This indicator will be available for all Italian and foreign bulls used in Italy and with at least 80% of reliability.

13. GENOMIC EVALUATION

Since December 2011 genomics (the branch of molecular biology that studies the genome) has become a new selection tool in the hands of breeders of the Italian Holstein. Previously the methods used to estimate the genetic value of commercial animals were based (and still are for those that are not using genomics) on two types of information:

- a. phenotype data (observable performance of daughters)
- b. pedigree (sire, dam and progeny)

With the invention of new DNA analysis technologies it is now possible to "know" a part of an individual's genes (genotype), estimate its value and calculate a genomic index.

This new methodology can be used to:

- 1. estimate the genetic value of a young animal with greater reliability than a pedigree index;
- 2. reduce the generation interval;
- maximize the choice of animals to be sent to progeny tests and/or to be used as sires of bulls/cows;
- 4. increase the reliability of bulls undergoing testing when the number of daughters is limited.

In short, a genomic index is calculated as follows:

- 1. genotyping individual animals;
- 2. collecting phenotypes (traditional genetic indexes EBV);
- 3. estimating the value of the individual markers (SNP) which determines the genotype;
- 4. calculating the direct (DGV) and overall (GEBV) genomic index.

On an international level, ANAFIJ is now officially accredited with ICAR, for relative's assessment. Moreover, ANAFIJ joins with Interbull GenoEX-PSE project to share bulls and cows' markers in order to assess international bulls' offspring.

13.1. Data

The data used, refer both to the genotypes of the bulls and to their traditional genetic indexes and is used as the input phenotype for estimating the effects of individual markers. The genotypes were collected thanks to ANAFIJ's collaboration on two research projects (SelMol and Prozoo), a cooperative agreement with four AI centers in Italy, exchanging material with certain European countries (Ireland and Switzerland), and an international agreement with North America (USA and Canada), United Kingdom and Switzerland. Several others have provided genotypes as well. Over 260,000 genotypes are now available. Genomic data available (May 2019) is shown in the following table:

Genomic Data	Number
Total genotyped bulls	206.897
Total genotyped heifers/cows	58.691
Training population	33.932

It must be noted that the number of genotyped animals is continuously increasing, since AI centers and breeders are continuing to collect samples from calves so that they can preselect the best animals.

13.2 Statistical model

In order to calculate a genomic index, one must estimate the value of the individual markers (currently more than 68,000) which make up the genotype of the individual. This estimate assumes that a mathematical model is used, which starts from the traditional indexes of a group of highly reliable (proven) sires and provides estimating equations based on individual markers. These equations can be used to calculate the genomic value of animals that are young or without progeny. The estimation process can be summed up as follows:

- 1. the input data is cleaned (control of sex, call rate and parents);
- 2. the reference population is identified (proven sires);
- 3. the effects of markers are estimated using the traditional EBV values of the proven sires;
- 4. the genomic index of all the animals is calculated

The model used at ANAFIJ to estimate these effects includes not only the 68,000 markers obtained after initial editing (cleaning) procedures, but also a so-called "polygenic" effect, which is entered in the model through the kinship information of the animals. The part of the variability not explained by the genetic markers can be "recovered" by incorporating kinships between the animals into the model:

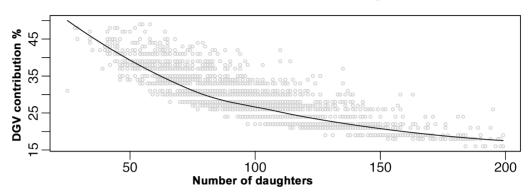
DGV = Markers + Classic kinship + error

13.3 Genomic index for bulls

A direct genomic index can be calculated both for sires with daughters and for bulls without daughters (young bulls) after the value of the individual markers has been determined. For sires with daughters, direct genomic information is combined with the traditional index to increase reliability. The final result is the GEBV. The weight of the two indexes (direct genomic and traditional EBV) depends on the number of available daughters: as the number increases, the weight of the direct genomic index decreases. This correlation can be observed in figure 1, where the weight of genomics as a function of number of daughters is reported for the GPFT.

Figure 1: **Proven sires.** Contribution of the direct genomic index to the GEBV as a function of number of daughters.

Proven Bulls - DGV contribution to gPFT



For young bulls (a young bull has no daughters, so no traditional index is available), the direct genomic index (DGV) obtained directly from the model without combining it with other information is used.

13.4 Genomic index for cows

The same approach as for male genomics can also be applied to female selection. Genomic indices of all traits can be calculated except for calving ease, mastitis and milk ability. Direct genomic breeding values (DGV) are estimated on the basis of the markers and pedigree (polygenic effect).

For cows (animals with information on their performance), are combined the traditional index (IGV) and those calculated using DNA analysis (DGV). Their traditional index (IGV) is not based only on pedigree but also on their performance (lactations, score, etc.). The weight of the two indexes (direct genomic and traditional EBV) depends on the number of lactations: as the number increases, the weight of the direct genomic index decreases. This is still lower than what happens with the proven bulls. In fact, the conventional indexes of the cows have less information than the proven bulls, for which the reliability, and therefore the weight is much lower. In practice, while for proven bulls their genomic index is similar to that calculated with the daughters, in cows major differences between IGV and genomic index are found. For young animals, of which there is no available information on their performance or their offspring, the direct genomic index (DGV) is used.

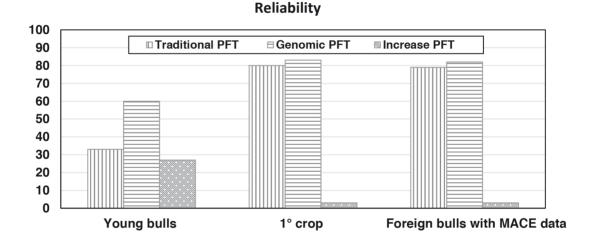
Summarize:

Indexes	Proven bulls	Heifers/Calves	Cows
Traditional Index (IGT/IGV)	Pedigree + daughters phenotype	Pedigree	Pedigree + phenotype
Direct Genomic Index (DGV)	DNA + Pedigree	DNA + Pedigree	DNA + Pedigree
Genomic Index GEBV)	DGV & IGT	DGV	DGV & IGV

13.5 Reliability

One of the greatest advantages of genomic evaluation is the superior reliability of the indexes obtained especially when bulls without daughters are considered. If a young bull is evaluated only using a pedigree index, his reliability rarely exceeds 35%. Using genomics, indexes can be obtained with a reliability of approx. 65-70%, which is approximately double the value obtained with the classic method. For cow it is the same, genomic index have a reliability around 60-70%. A newborn heifer with DNA analysis has about the same reliability as a cow with three lactations. This sharp increase of reliability, compared with the traditional pedigree that has about 30% of reliability, should push to increase more the number of heifers used as potential bull dams. Observing the national selection index (PFT) in figure 2, one can see the difference in reliability between traditional evaluation and genomic evaluation, both for proven sires (Italian or foreign) and for young bulls.

Figure 2. Reliability between traditional and genomic evaluation. National selection index (PFT), both for proven sire and for young bulls.

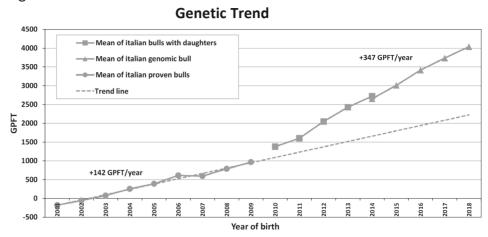


In young bulls the average increase in reliability for PFT is approx. 25%, but it is lower for proven sires. This result is to be expected because it shows that genomics adds little for sires with daughters, since the data for estimating them is provided by their daughters.

13.6 Genetic trend

Trend of GPFT in recent years has had a considerable increase; in the graph of GPFT it is easily seen that the increase of GPFT 2009-2018 is significantly higher than that of the pre-genomic era.

Figure 3. **Genetic Trend gPFT**. The impact of genomics on the breed selection program; it can be seen clearly the change of the genetic trend and the generation interval.



Proven bulls born before 2009 were selected without the use of genomics, while in 2010 the genomics start to have an impact on the system. A trend line dashed is shown to highlight what would be the genetic trend without the introduction of genomics. Looking at the period 2009-2018, it can be noted that:

- The dashed trend line shows the genetic progress of 142 points per years;
- Line marked with squares depicts Italian bulls with daughters, it proves the great results achieved by genomics;
- Since genomic analyses have started, a genetic progress of +347 points / year
 is depicted. The difference between the line marked with triangles and the
 dashed trend line shows that the use of genomics has led to an extra increase
 of GPFT of 205 points. It means that genomics has added 144% extra genetic
 progress.

13.7 Haplotype and genetic factors

Genomics is continuously evolving and very often new DNA chips are added. New genetic disorders and important economic traits have been discovered in this way. Thanks to DNA analysis and genealogy it is possible to see which animals are carriers of these traits. In recent years, we have given particular attention to the haplotypes (DNA segments) and to genetic factors. When the causative gene is unknown, a DNA segment is used, while when the causal gene is known a direct test on the genetic factor is applied. In the case of genetic factors only one or two markers with a functional impact are used and not a group of markers, such as for the identification of haplotypes.

The availability of these on DNA tests enables:

- Monitoring the presence of traits (desired or undesired)
- Exploit desired traits
- Diminish the presence of undesired traits or prevent them from dissemination
- · Avoiding risky matings
- Avoiding investments in young bulls with undesirable traits or with genetic defects.

In this manner AI centers can reduce risks in making investments of bulls which later may manifest some of these genetic defects. While the advantage for farmers is to reduce and / or avoid the health problems in their breeding. In this way genomics is providing added value to farmers.

The table shows all the genetic traits currently under control. Since November 2015 the direct tests were added to markers that determine genetic factors, such as K-casein.

Genetic traits now monitored

Trait	Lethal	Effect
Reproduction	Y	Brachyspina, HH1, HH2, HH3, HH4, HH5, HH6, BLAD, Citrullinemia, CVM, DUMPS, Spinal muscular atrophy, Spinal demyelination, Congenital muscular dystonia 2, Maple syrup urine disease, Osteopetrosis
Feet	Υ	Mulefoot
Horns	N	Polled
Survival	Υ	Deficiency of cholesterol, Dilated-Cardiomyopathy, Factor XI, Ehlers-Danlos syndrome
Hair	N	Red recessive, Red dominant, Telstar, Silver color dilutor
Milk	N	Alfa S2-casein, Beta-casein AB, Beta-Lactoglobulin, Kappa-casein AB, Kappa-casein ABE, Kappa-casein ABCE

Since February 2016, ANAFIJ has added an imputation process (an estimation of missing genotype segments using information from animals with the same DNA strands) also for genetic deficiencies to have information about genotyped animals whose DNA panels do not include these factors. Thanks to this process, the genetic factor information is revealed for genotyped animals with different DNA panels (i.e. panels without information about these factors). It is important to emphasize that for DNA panels without genetic factor information any tests are available to directly identify them; however, thanks to the imputation process it is possible to trace DNA segment transmissions which reveal these genetic factors. Nowadays, information about these deficiencies are available for at least 300,000 animals. For each tested animal, the result specifies if it comes from a direct test or from the imputation process. Results from imputation are unofficial, i.e. they are not published but they are available for AI centers and for mating programs.

14. THE INTERNATIONAL PROOFS

14.1.Data

Input data for International genetic evaluation are the Official Breeding Values computed in each country participating in the service. The service of international evaluation is run by International Bull Evaluation Service (INTERBULL) in Sweden. All countries, including Italy, are sending their official proofs to this center at a given date determined by a calendar of publications approved each year. All breeding values for bulls with at least 10 daughters in 10 herds, are sent to INTERBULL, while for calving ease the limits are bulls with at least 50 daughters in 10 herds. For test day model data the proof sent to INTERBULL is the one for first, second and third lactation weighted according to number of test days. In April 2015 the traits for which an international evaluation was provided were: milk, fat, protein, standard conformation traits, calving ease direct and maternal, somatic cells, fertility and body condition score.

14.2. MACE MODEL

Each trait is evaluated by INTERBULL with a Multiple Across Country Evaluation (MACE in brief): in practice the proof in each country contributing to the evaluation is considered as a separate trait. In this way it is possible to compare bull rankings across countries. The difference across countries is measured through genetic correlation among the various countries. The effects accounted for in the model are the <u>country average</u> and the <u>genetic groups for the maternal grand dam and for the material grand sire</u> in case they are born before 1986. Table shows the genetic correlations (April 2019) between Italy and some of the participating countries for all the traits for which the international evaluation is provided.

Traits							
Country	Protein	Туре	Somatic cells	Mastitis	Longevity	Fertility	Calving ease
USA	0.88	0.84	0.89	0.88	0.74	0.91	0.75
CAN	0.85	0.81	0.90	0.87	0.75	0.85	0.77
FRA	0.84	0.87	0.93	0.88	0.60	0.84	0.76
DFS1	0.86	0.81	0.92	0.88	0.65	0.84	0.77
NLD	0.83	0.79	0.88	0.88	0.54	0.85	0.77
DEU	0.83	0.82	0.94	0.89	0.74	0.91	0.76
AUS	0.68	0.55	0.86	0.86	0.54	0.78	0.70
NZL	0.69	0.56	0.85	0.85	0.44	0.59	0.76

¹DFS = Denmark, Finland, Sweden

14.2.1 How it works

When a bull has a proof only in one country the model transfers, using the available information about genetic correlation and difference in scale in another country, the daughter deviation i.e. the bull proof once we subtract from it the parent average. The final proof of the bull in the other country is then computed adding to the rescaled deviation the parent average in the new country. When a bull has daughters in more than one country the deviations are weighted by the number of daughters and then summed together before re-adding the pedigree in the country of interest.

14.3 GMACE model - Young bulls

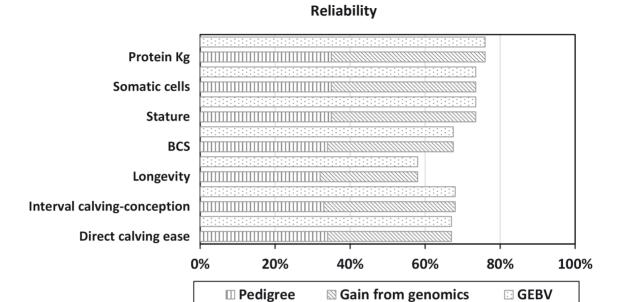
With the arrival of genomic evaluations in various countries, there has been a growing interest and demand for the international marketing of semen of young genomic bulls. This creates the same problem that originated before the development of MACE. The fact that proofs for progeny proven sires are not comparable across countries also extends to genomic young sires. For this reason, INTERBULL launched from August 2014 a new official service, referred to as Genomic MACE.

This process is called GMACE (Genomic Multiple Across Country Evaluation). Genomic MACE evaluations use the national genomic evaluation estimated in each of the countries where a genotype exists and produces a genomic evaluation on the scale of the country that does not yet have a genotype. This service will help the Italian breeders to assess the genetic merit of young bulls belonging to countries not part of the Intercontinental consortium (United States, Canada, Great Britain, Italy and Switzerland) who have directly a national evaluation.

14.3.1 How it works

Every country that wants to participate in the GMACE evaluation sends to INTERBULL national genomic indices for all the traits, for which it has passed the genomic validation. Currently Italy has validated all the traits for which it is already published the official national genomic index (production, morphology and functionality). Clearly the ideal scenario would be to have a direct exchange of genotypes among all countries, as is already the case within the countries belonging to the Intercontinental consortium (United States, Canada, Great Britain, Italy and Switzerland) and EuroGenomics (France, Scandinavia, Germany, Spain, the Netherlands/ Flanders and Poland). Estimation of genomic breeding values, starting directly from the genotype of each animal is much more reliable than a pedigree index, especially for young bulls (no available info on their daughters) or when the number of daughters is limited. Figure 6 shows the level of the reliability of pedigree and genomic indices in different groups of traits.

Figure 4. **Reliability of pedigree index and genomic index**. Different traits estimated from genomic data-base of ANAFIJ



14.4 What is published

INTERBULL computations give back to each country the breeding value of all bulls evaluated in the world (around 70,000 bulls) on the country scale. Each country has approved rules of publications of those proofs. These rules are subject to changes and the up to date rules are always published on the website in the area related to breeding values. In August 2007 in Italy, the rules of publications were as follows:

PRODUCTION – CONFORMATION – LONGEVITY – FERTILITY – CALVING EASE: INTERBULL proof is official only for foreign bulls.

GENOMICS GLOSSARY

Allele: a particular DNA variant that may be responsible for alternative forms of a trait. Every individual has a paternal allele and a maternal allele, which may be the same (homozygote) or different (heterozygote).

DGV: direct genomic index (the simple sum of the effects of markers).

DNA: (deoxyribonucleic acid). The substance that makes up the gene pool. It is formed by a sequence of 4 nucleo bases.

EBV: traditional index.

Genetic factor: genetic variant where the gene is known (milk caseins).

GEBV: the combination of a traditional index obtained using information on daughters (EBV) and a direct genomic index (DGV).

Gene: the basic physical unit of heredity. A segment of DNA that codes for a function or several correlated functions.

Genome: the set of chromosomes contained in every cell in an organism.

Genomics: the field of molecular biology that deals with the study of the genome of living organisms.

Genotyping: The process of determining a genotype by analyzing DNA. A specified sequence is compared with another. SNP genotyping measures genetic variations (alleles) in the polymorphism of a single nucleotide (SNP).

Genotype: The set of genes that make up the DNA of an organism or population.

Haplotype: DNA segment strictly linked. It can pass from parent to offspring

Imputation: Methodology used to estimate missing parts of a genotype using information from individuals in the same family.

Molecular markers: Pieces of DNA whose alleles can be easily identified in the laboratory. They are used to identify neighboring genes.

Nucleotide: The building block of DNA.

SNP molecular marker panel: A laboratory kit used to determine the genotype of a certain individual (genotyping). These kits may contain different quantities of information. Moreover in these panels are also included markers which have effect on known genes (ie: milk proteins).

Polymorphism: The simultaneous presence of two or more alleles or nucleotides in the same population.

SNP: single nucleotide polymorphism. Polymorphism in a specific base or nucleotide in the DNA sequence; for example, at a point in the DNA sequence where one allele has one A and another allele has one T.

Genetic Evaluation

ANAFIJ

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