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Female Fertility in French Dairy Breeds: Current Situation and Strategies for Improvement

Anne BARBAT¹⁾, Pascale Le MÉZEC²⁾, Vincent DUCROCQ¹⁾, Sophie MATTALIA²⁾,
Sebastien FRITZ³⁾, Didier BOICHARD¹⁾, Claire PONSART⁴⁾ and Patrice HUMBLLOT⁴⁾

¹⁾INRA GABI, 78352 Jouy en Josas Cedex, ²⁾Institut de l'Élevage, 149 Rue de Bercy, 75595 Paris cedex 12, ³⁾UNCEIA 78352 Jouy en Josas Cedex, ⁴⁾UNCEIA R&D, 94704 Maisons Alfort Cedex, France

Abstract. In most countries where genetic selection for milk production has been conducted, a continuous degradation of reproductive performances has been observed in dairy cattle. This paper describes the phenotypic evolution of fertility traits in France for the main 3 dairy breeds and the different actions undertaken to counteract this unfavourable evolution of reproductive performance. In 2001, functional traits (including conception rate as a fertility trait) have been included into the French total merit index ISU, to take these traits into account in the selection objective. In addition, a marker-assisted selection (MAS) program has been developed since 2001. Through this approach, a set of QTL related to fertility traits were identified using the phenotypic information for fertility collected on a national basis. Fine mapping of these QTL led to their introduction in 2008 in genomic evaluation and the routine selection for fertility traits in breeding schemes. This approach will evolve in the near future through the inclusion of results of several genomic research programs that were conducted either in station or under field conditions aiming at identifying genes and pathways controlling cattle fertility (based on genomics, transcriptomics and proteomics) and at enhancing phenotyping for reproductive performance. The paper describes the general approach behind these evolutions, the expected benefits of genomic selection and first results. Information on the potential use of reproductive technologies that may improve the efficiency of multiple trait genomic selection are also presented.

Key words: Dairy cattle, Fertility, Genetic selection, Phenotyping, Reproductive genomics

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Dairy cow fertility is a major challenge for farmers and all partners involved in cattle breeding and selection. Inheritance of fertility and its relationship with dairy production has been of a major concern for a long time [1–3]. The situation is even more critical today as since the 80's, a strong degradation in female fertility has been observed in all dairy breeds especially in the Holstein breed [4]. This phenomenon is not specific to France but to all dairy producing countries [5–9]. One of the reasons is genetic selection for milk production because of the negative genetic correlation evidenced between milk production traits and fertility traits [10]. In France as in many countries, tools based on quantitative genetics have already been implemented routinely to better control the genetic level of reproduction traits. Since 1998, INRA has developed a genetic evaluation on female fertility together with other functional traits and since 2001 a total merit index (ISU) which includes fertility in breed selection objectives is routinely used. More recently, a Marker-Assisted Selection program (MAS) which has been implemented by INRA, LABOGENA and UNCEIA aims at improving selection effectiveness, in particular for low heritability traits (such as fertility).

This article aims on the one hand to describe the evolution of fertility traits at the phenotypic and genetic levels and on the other hand to present the work undertaken by INRA, UNCEIA and IE (Institut de l'Élevage) on this topic, especially in the field of genomic research. The potential use of combined reproductive technologies to improve the efficiency of multiple traits genomic

selection will also be discussed.

Phenotypic Trends

Data

AI data (dates of artificial insemination and dates of calving) recorded in the national Genetic Information System (GIS) were analyzed to study the phenotypic evolution of fertility in the three main dairy breeds in France between 1997 and 2006. These breeds are an international breed, the Holstein, and two national dual purpose breeds, the Normande and the Montbeliarde breeds. Only AI leading to parity 1 to 3 were included. For a given parity, results following AIs of rank 5 or more were excluded, as well as data from cows with unknown sire. Indeed, the distribution of cows with unknown sire within age class, parity and region differs from the one of cows with known pedigree. They usually exhibit lower fertility results but it is not possible to determine whether this is due to their own reproductive performance or to poor recording of reproductive events. The phenotypic trends presented here are based on more than 37 million AI.

Evolution of conception rate after each AI

The AI status (a 0/1 trait indicating whether or not AI is followed by a successful pregnancy) is determined from AI dates and calving dates based on a number of rules using the information on the existence of a following calving of course, but also the history of all AIs between two calvings (or before first calving for heifers)

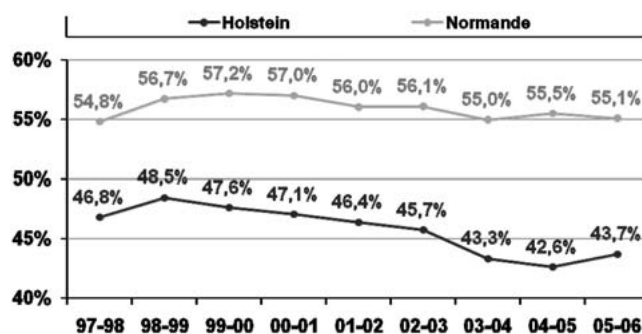


Fig. 1. Phenotypic evolution of conception rate in Holstein and Normande cows over years (97-98 to 05-06=campaigns of AI; From Barbat *et al.*, 2008 [4]).

and the average gestation length for the breed. The result of many AIs is obvious, for example when AI is followed by a calving after a period corresponding to the gestation length or when AI is followed by another or several other AI. In other cases, the result is much less clear, for example when the inseminated cow is sold to another farm or when AIs have been made recently. In the latter case, the 0/1 result is replaced by a probability of gestation, calculated from the interval between AI and current day, cow parity, breed and AI number. However, only AIs with a result 0 or 1 were kept in the following analysis.

The overall conception rate in the Holstein breed is now about 40% (Fig. 1). The decline in fertility is observed in the 3 breeds and has become especially perceptible since 2000.

For the Normande and the Montbéliarde breeds, the extent of this decline has been about 2% to 3% since this date, while in Holstein, it has reached at least 5% for cows (Fig. 1).

After the period 1999-2004 where degradation was over 1% per year, the last three years display a more stable trend. For the three breeds heifer conception rates declined also by 1.5-2% and this trend was particularly visible during the 2000-2004 period. To increase conception rates, it is generally recommended to wait for 50 days after calving before practicing AI. During years 1995-1997, such information was widely spread to farmers and contributed to reduce the number of females inseminated too early after calving. From this set of data, the pregnancy rates are decreased by 6 to 8% when AIs are performed before 50 days post-partum. Beyond 60 days, the conception rate after 1st AI becomes independent from the interval since calving and reaches 54% in Montbéliarde, 51% in Normande and 39% in the Holstein breed respectively.

Evolution of Intervals

The average interval between calving and first AI is related to cow's ability to reinitiate ovarian activity and become cyclic. This interval did not significantly change over the last decade: for the Montbéliarde breed, it remains around 75 days and varied between 76 and 78 days in the Normande breed. Due to fewer AI performed before 70 days, this interval is longer and increased from 84 days to 88 days in the Holstein breed.

The interval from calving to conception remained almost con-

stant in the Montbéliarde and Normande breeds. By contrast, the deterioration of reproductive performances in the Holstein breed is unambiguous: in 2005, the average interval from calving to conception reached 128 days, an increased by 13 days over nine years. This deterioration is also evidenced through the increase from 23 to 28% of the percentage of Holstein cows inseminated more than 3 times. By contrast, this percentage remained rather stable for the other breeds and for heifers.

During the last decade, Montbéliarde cows have maintained a constant calving interval of around 386 days and this interval is only 2 days longer in the Normande breed. Due a greater number of unsuccessful AI and despite a shorter gestation length, Holstein cows showed in 2006 an average calving interval of 408 days, i.e., 20 days more than for the 2 other breeds and 13 days more than ten years earlier. However, these results must be connected to the production systems: in western areas where most cows are Holstein, a proper management of seasonal reproduction is less essential, because grass and forages are mostly available all over the year and the pressure to get one calf per cow per year, particularly from high producing cows is not so strong when compared to other breeds.

Regional and seasonal factors

Reproductive performances vary strongly between regions. For instance, in 2006, conception rates ranged from 36 to 57% depending on districts. A given local situation is the result of a combination of multiple effects. For example, in regions where the proportion of Montbéliarde cows is important, where calvings are grouped mostly in fall and production is not too high, the fertility results are much better than in areas in which the Holstein population is dominant and where AIs are distributed all year round. The feeding systems and level of intensification also play a role in causing these differences.

More AIs are performed in winter than in summer and pregnancy rates are higher during the most intensive period for AI activity. Generally, when calving are distributed all year round as in Western France and in contrast to Eastern and North Eastern France, fertility results are poorer because a large proportion of cow AIs are performed during a less favourable period (spring). As a consequence, a large part of the regional differences in conception rate results from the influence of management and seasonal variation in the distribution of AI.

Genetic Evaluation

Data

The genetic evaluation of fertility carried out by INRA is based on the same AI data as above, coming from the national Genetic Information System (GIS) including all cows that are recorded for milk production. The evaluated trait is conception rate after each artificial insemination. It is a repeated trait over the cow's career. All AIs are included in the evaluation, and this improves the reliability of estimated breeding values (EBV) for this trait for which heritability has most often been found very low [1, 3, 11-13]. Currently, 30.5, 4.8, and 4.3 million AI are included in each evaluation for the Holstein, Montbéliarde, and Normande breeds, respectively.

General strategy

Only conception rate to each AI is currently evaluated in France. An evaluation on ability to recycle will be routinely evaluated starting in June 2010.

The heritability of conception rate is very low (1–2%). As a result, selection for this trait using a standard genetic improvement approach is difficult [12, 14]. However, as shown in former studies [2, 15–17] genetic variability is high and it is possible to detect extreme sires with differences in conception rates of their daughters of $\pm 15\%$ or more. Fertility of dairy bulls is currently evaluated only from the results of their daughters because the genetic correlation between female and male fertility is close to 0 [12, 14]. However, heifer and cow fertility are two different traits with a genetic correlation of about 0.60, i.e., with a partly different genetic background. Although both traits are genetically evaluated, only the “cow” fertility index is currently published. In the near future, a fertility index combining conception rate in heifer and adult cows and ability to get cyclic during the post partum will be introduced. Since June 2007, the evaluation model [18] used is an animal model in which the breeding values of males and females are estimated at the same time taking into account the entire pedigree.

In practice, the genetic evaluation of fertility is performed in two steps. First, a univariate evaluation is performed separately for heifer and cow. AI and performances are corrected for all non-genetic effects accordingly. These corrected performances are then included in a multiple trait evaluation [19], which optimally merges information from fertility traits with other functional traits, accounting for the nonzero genetic correlation between them. The resulting fertility EBV (among other EBV for other traits) which is published includes information on the trait (heifer or adult cow conception rate to each AI) as well as information on predictor traits (longevity, type traits).

Univariate genetic model

The univariate models for heifer and adult cow conception rate at each AI include random effects (additive genetic effect and permanent environment effect of each female, interaction between service bull (the bull used for AI) and year) and fixed effects reflecting many interactions (Herd-year, AI technician-year, Month-region-year, Day of the week-region-year, Breed of service bull, Age at AI for heifers, Interval from calving to insemination-parity for cows, parity for cows, and inbreeding of the embryo and of the cow). The distribution of additive genetic effects includes relationships between all animals. The other random effects are regarded as independent. In particular, relationships between service bulls are ignored. The heritability and repeatability of the trait are 2 and 5% respectively. The proportion of variance of the service bull-year effect is 1%.

Multitrait genetic evaluation

The above described evaluation provides basic EBV for “Heifer fertility” and “Cow fertility” as well as estimates for all other effects in the model. However, these univariate fertility evaluations are likely to be biased for two reasons. First, inseminated cows are a selected sample of all cows since heifers that remain non-pregnant have been culled. This causes a bias on “cow fertil-

ity” EBV. Second, selection also occurs after first calving, particularly on production traits which are known to be negatively correlated with fertility. Ignoring these selection steps would lead to an overestimation of fertility EBV. The multiple trait evaluation re-analyses jointly these different traits, accounting for these genetic correlations [19]. To simplify computations, adjusted records (i.e., records corrected for all non genetic effects and averaged at cow level) are used. In the multiple trait model, twelve traits are combined (conception rate for heifer and cows, other functional traits such as somatic cell count, functional longevity, milking speed and 6 type traits (chosen within breed) which are used as predictors of functional traits. Milk production is also included because it is strongly selected and negatively correlated with fertility and other functional traits. The relevant type traits for the Holstein breed are Angularity, Rump Angle and Body Depth. Deep, angular cows with horizontal rumps are found less fertile than narrow, round cows with steep rump [18].

The EBV from the multiple trait evaluation are finally combined into the total merit index (called ISU) with, for the Holstein breed, an economic weight of 1 for the overall production EBV and 1/4 for each of somatic cell score, fertility, longevity, and overall type EBV, respectively. Indeed, this is another important benefit of the approximate multiple trait approach [19]. The optimum weights for the total merit index are the economic weights. They do not have to be modified to account for correlations and differences in reliability between traits. The resulting TMI is defined in the same way for males and females and can be used to compute pedigree TMI.

As other functional traits in France, heifer and adult cow conception rate EBV are expressed in genetic standard deviation units. The genetic variability of both fertility traits is 7%. A difference of one EBV unit between two bulls means an expected conception rate difference of 3.5% of their daughters.

Genetic trend

The genetic trend for conception rate has been measured by the average EBV of cows by birth year. After a small decline for females born between 1998 and 2002, the genetic level for fertility of Montbéliarde cows stabilized at the same time as the pregnancy rate observed for the same females following first calving. In the Normande breed, a small increase in mean EBV started in 1999, slightly before the increase in pregnancy rates of primiparous cows which was also influenced by other non-genetic factors. However, these genetic trends remain modest: 0.15 genetic standard deviation lost between 1996 and 2001 in the Montbéliarde breed or gained between 1998 and 2002 in the Normande breed are equivalent to 1% difference in conception rates.

In the Holstein breed, phenotypic and genetic changes are more noticeable (Fig. 2). A decrease of more than 4% in pregnancy rate was observed between primiparous cows born in 1993 and those born in 2002. The difference in genetic level for “cow fertility” between these two generations is slightly above 0.2 genetic standard deviation, which corresponds to a 1.5% loss in conception rate. In this breed, the genetic contribution to the degradation observed over the past years on pregnancy rates of primiparous cows would therefore be approximately 35 to 40%. The genetic

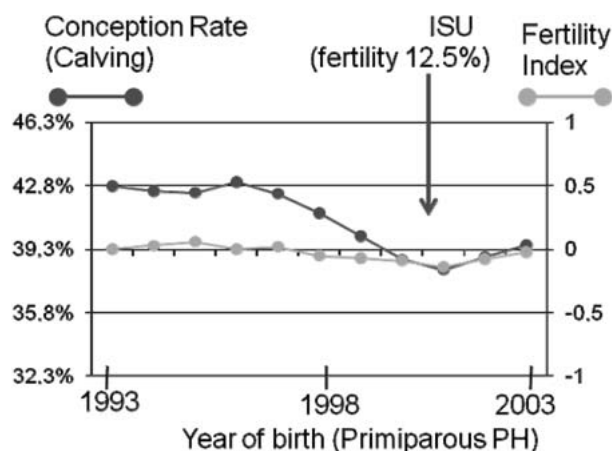


Fig. 2. Calving rates and fertility indexes of primiparous Holstein cows (From Barbat *et al.*, 2008 [4]).

trends of “Heifer fertility” follows the same trends as those reported for cows but with a smaller amplitude. The recent positive (or flat) trends need to be confirmed in the near future and may result from a better use of fertility EBV in the selection objectives and selection schemes of these breeds.

Research in the Field of Reproductive Genomics

More than five years of research have been conducted to identify genes that control fertility in the bovine species. Through a first approach the structure of the genome was studied by using mapping work. Another type of work aimed to specify the function of these genes involved in reproductive success. All this work has been carried out within the framework of the program GENANIMAL (Program funded by the ANR and professional structures constituting APIS-GENES).

QTL fine mapping and accuracy of phenotyping for fertility traits

The Department of animal genetics of INRA (DGA/GABI) has undertaken in collaboration with UNCEIA the identification and refine mapping of fertility QTLs in dairy breeds. A first primo localization design based on a set of 169 markers covering whole genome resulted in the detection of several QTL affecting reproductive traits [20]. However, the accuracy of localization was moderate. In a second phase fine mapping that intended to specify more precisely the location of different QTLs was performed. This step required the addition of individuals to the design together with a densification of the set of markers. Before 2006, the work of QTL’s refine mapping required a huge amount of work for typing and efforts focused on few QTLs. This allowed to study a fertility QTL respectively on chromosome 7 and three fertility QTLs on chromosomes 1, 2 and 3. Twenty tags were added on each chromosome and 17 additional families (on top of the 9 initial ones) were studied in this design. Since 2006 and the development of high throughput technologies, refine mapping of existing QTLs is easier

and it is now possible to add thousands of markers at a reasonable cost.

A first partnership with the National Centre for Genotyping (CNG) helped to clarify the position of chromosome 3 QTL in a region that contains a dozen of genes. Through this design, 1643 bulls from 3 breeds have been genotyped with a set of 1536 Single Nucleotide Polymorphism markers (SNP) [21]. Additional work has been conducted through the CARTOFINE project [22] where 3200 bulls from three breeds have been genotyped on 54,000 SNP markers. This project enabled to refine the mapping for all QTLs presently included in the Marker-Assisted Selection program and especially fertility QTLs. The achievement of a large number of genotyping is no longer a limiting factor in such studies. The work of QTL fine mapping now requires an important and precise collection of phenotypes. For fertility, performance is measured mainly by AI success which includes many different reproductive traits. As mentioned before, this character is very much influenced by environment and has a low heritability. However, by measuring the phenotypes with more precision, one can expect to improve the accuracy of the detection and the identification of candidate genes. A first attempt in that direction was made through work achieved at INRA by recording the results of AI success at different intervals to check if the effect of a given fertility QTL was pronounced more or less early during pregnancy. This first work demonstrated that chromosome 3 QTL affected preferentially fertility events before 90 days of gestation whereas for other QTLs (chromosome 7) the effects were seen on events beyond 90 days after AI [23].

This first approach to refine fertility phenotypes only by using non-return in estrus data is still not very precise and another project (GENIFER; [24]) aimed to use more precise phenotypic data associating non-return rates and results of hormonal assays. All QTLs and all candidate genes associated with fertility identified through the CARTOFINE project were studied. The design relies on daughters from 12 sires that have been genotyped for fertility associated markers and aimed to clarify the action of each QTL or each candidate gene. The phenotyping of more than 3,500 females has been achieved and information has been recorded from the corresponding herds. Refined phenotyping was performed by the use of hormonal measurements allowing to determine if the cows were inseminated at the right time (progesterone at day of AI) and providing a precise status of gestation at different stages between the date of AI and 90 days post-IA. Individual genotypes will be analyzed with the corresponding phenotypes (late or early embryonic mortality, late abortions, pregnancy). The first results show that the fertility of the groups of females issued from homologous fathers (+/+ or -/- for the fertility QTL on chromosome 3) express very different and even extreme fertility results in terms of pregnancy rates and early embryonic mortality (Fig. 3) when compared to other groups. When considering all groups, pregnancy rates were negatively correlated with early embryonic mortality (i.e. before Day 16 post fertilization, [25]). Only one group of daughters expressed a high rate of late embryonic mortality which is in accordance with earlier findings [17]. The tools developed and the results of this work will be used in other genomics programs aiming to study more precisely the effects of genes.

In parallel with the work made in female reproduction, another

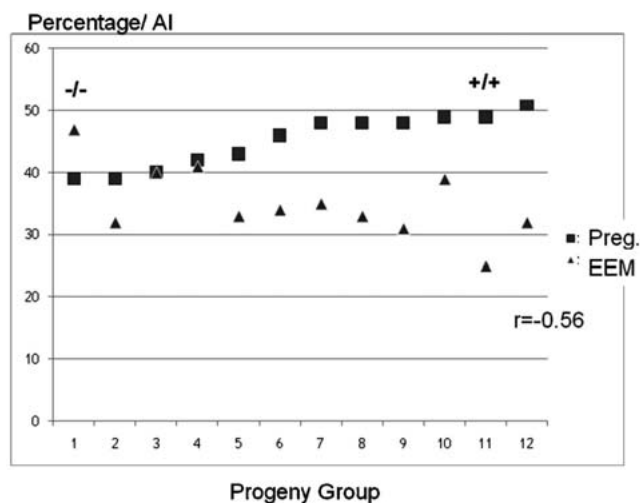


Fig. 3. Negative relationship between pregnancy rates (Preg.) and early embryonic mortality rates (EEM) from 12 progeny groups. Females from groups identified “+/+” and “-/-” are born from homozygote bulls for this fertility QTL.

program aimed to primo-localize QTLs in relationship with male reproduction by phenotyping individuals on semen production and fertility variables. This work done under collaboration between UNCEIA, INRA PRC Nouzilly and GABI allowed to identify several chromosomal regions associated with such variables [26].

Gene expression

Several programs aimed to investigate relationships between gene expression and tissue function involved in reproduction from years 2003 to 2007.

There is now a lot of information in the literature that demonstrates the critical roles of oocyte quality and function at various stages of growth and meiosis in determining very early embryonic development [27–29]. It has been shown for some time that specific genes expressed during oocyte growth affect directly very early stages of embryonic development. Knockout experiments performed initially in mice demonstrated their critical role in embryonic development and there is now evidence for their presence and role in the bovine [30–32]. In addition to these specific genes the role of many genes involved in the control of multiple functions within the oocyte and the embryo have been reported [33]. The pattern of expression of the genes and accumulation of RNA transcripts during oocyte growth and maturation is really essential for early embryonic development as RNA's and proteins accumulated within the oocyte at these early stages are used by the young embryo before the activation of his own genome [34]. On top of this, the oocyte brings components of the developing nucleolus which are necessary for rebuilding the RNA machinery and expression of the embryonic genome [34]. All those consequences of oocyte quality and function on early embryonic development have very strong implications for ART technologies and more generally for the control of fertility and probably explain the effects of many factors on fertility and embryo production efficiency.

As a complement to those former studies performed in the mouse and cow, the program OVOAGENAE aims to develop specific tools to reveal the activity of oocyte genes that may be related to embryonic development [35]. The first part of the work confirmed the importance of candidate genes oocyte specific previously identified in cattle or other species. In addition, many new sequences corresponding to oocyte genes have been identified. RNA differential expression is currently measured from oocyte material at different stages of development and from different physiological status (mature vs. immature or from pre pubertal or post pubertal females). The tools developed will be used also to study potential differences in gene expression between females identified as fertile or unfertile in terms of embryonic development both *in vivo* and *in vitro*. Advantage will be taken from the identification of females with extreme performances. The superovulation model associated either to embryo collection *in vivo* or to Ovum Pick up followed by In Vitro Fertilization and culture allowed to identify individuals expressing repeatedly different rates of transferable blastocysts (range from 15 to 50% or more; [36]).

Another set of programs aimed to study the relationships between the expression of somatic genes in reproductive tissues and fertility. In a similar way, specific tools were developed to study those relationships in the oviduct, uterus, corpus luteum and liver around time of implantation (programs GENSOM and EMBRYOGEN) in cows submitted to different regimes. Expression was compared in relation with pregnancy occurrence and in relation with the metabolic status induced by nutrition. Preliminary results show that the expression of genes that reflects the metabolic status is altered mainly in the uterus [37]. In addition, limited under expression of IGF1 and over expression of IGFBP2 in the liver creating different IGF1/IGFB2 ratio were found in relation with regime and pregnancy status (low ratio in non pregnant cows and in case of negative energy balance). This is consistent with the fact that nutritional effects may also affect the quality of interactions between the embryo and the uterus. It was recently shown that cows in severe negative energy balance had altered proportions of immune cells within the endometrium and that several components of the IGF system were involved in the control of tissue remodelling and repair within the uterus [38] inducing up regulation of immune related genes in cows with severe negative energy balance when compared to their counterparts. Many genes involved in protein modification and collagen catabolism (especially metalloproteinases) were also regulated by negative energy balance. Following those results, the reproductive tract may not fully recover following calving and so does not provide a suitable environment for early embryo development especially in case of strong negative energy balance [38]. Such processes involving tissue repair and immune response in the uterus may also be the source of embryonic mortality taking place by the time of maternal recognition of pregnancy [37, 38]. Such mechanisms may explain the relationships found between the BCS status of cows by time of calving and the occurrence of late embryonic mortality previously reported [25, 39].

In addition to those studies, another approach was to use the information taken from the comparison of congenic strains of mice with extreme fertility phenotypes [40]. Candidate genes have been

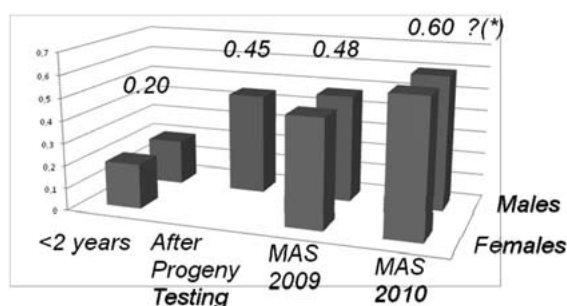


Fig. 4. Accuracy of fertility EBV of animals at birth and after usual progeny test without molecular information, and of animals at birth with MAS information (S Fritz *et al.*, INRA UNCEIA 2009, unpublished).

found and related to fertility phenotypes both in male and female mice. Comparative mapping and the potential relationship of those genes with fertility phenotypes are now under investigation in the bovine.

Conclusion

It is hoped that common research and application aiming to improve selection on bovine fertility will contribute finally to improve fertility performances especially in dairy cows. Results from the previous years show that, giving more weight to fertility in the synthetic index which represents major selection objectives, already induced improvements in reproductive performances and it is hoped that this will be confirmed in the near future. A better approach of risk factors and of their impact on reproductive performances through epidemiological studies may have also contributed to this result [41]. MAS for several traits including fertility is already used routinely and may reinforce the efficiency of selection for this trait as demonstrated by the expected gain in precision for genetic evaluation brought by its use (Fig. 4).

The use of reproductive biotechnologies and especially those based on in vivo or in vitro embryo production may also contribute to improve the efficiency of MAS especially when used for multiple traits [42, 43]. The effectiveness and the potential impact of these techniques for this purpose are under study.

It is anticipated that the beneficial effects expected from selection on reproductive traits will increase together with the use of numerous pertinent markers that will make this process more accurate and efficient. These effects should become perennial as soon as the results of these programs will be integrated in the routine selection process.

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