A wide range of tools to improve reproduction in dairy cattle

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Abstract

As most countries where genetic selection for milk production has been conducted, France has been faced a continuous degradation of reproductive performances in dairy cattle especially in the Holstein breed. A research and development programme conducted by UNCEIA and its partners has been proposed to face the decrease in reproductive performances observed in high producing dairy cows. Since 2002 several tools related either to genetics and environmental/management effects on fertility were developed in an attempt to stop this decrease or even improve female fertility.

New genetic tools associated with Marker Assisted Selection (MAS) progressed very rapidly those last years. MAS is based today on sets of thousands of genetic markers. This will result in a more rapid genetic progress, particularly regarding traits with low heritability or reliability, such as fertility. In parallel with those technological evolutions, attempts are made in different countries to strengthen the value of the genomic information by including more and more animals in the evaluation and selection process, as illustrated in 2009 by the creation of Eurogenomics which gathers the main breeding companies in Europe. The consequence of this is the calculation of more reliable estimates for the desired traits together with the preservation and or optimisation of the exploitation of genetic variability.

A second part of this programme was dedicated to farming practices. New field references on heat detection and insemination practices, body condition scoring were obtained from epidemiological trials. Moreover, herd follow-up methods were implemented to investigate reproductive herd management. The current use and impact of these different tools, which were developed with the participation of the local AI centres will be presented.

The first results originating from last years genetic and phenotypic evaluation tend to show positive trends for fertility. The combined use of new genetic evaluation and herd management tools, which were developed with the participation of the local AI centres should contribute to optimize reproductive performances in dairy herds.

Keywords: Fertility, genetics, breeding management, consultancy on farm, post partum.

1.0 Introduction

In France, as in many other countries fertility has decreased in the Holstein breed since twenty years. More recently, the data from the national data basis showed a decrease in reproductive performances in the two other main dairy breeds (Montbéliarde and Normande) between 1995 and 2005. Such a decrease was also reported too from heifer data (Barbat *et al*. 2005).

Conception and early pregnancy failures are linked to several management characteristics: feeding (assessed by body condition score), milk production, genetic potential, health state and season of reproduction (Humblot 2001; Grimard *et al*. 2006). Moreover, the sensitivity of the oestrus detection may be estimated to be around 50 % (Grimard *et al*. 2004). Evolutions of farm structures, social demand and perception of farming practices by the breeder may also influence the performances of reproduction.

Since 2002, the management issues have been approached through 3 main axes together with AI centres and "Institut de l'Elevage" (technical support organisation). The first aim is to actualise some references and advises given to farmers to manage reproduction. A second part of this work is dedicated to the methods of herd follow-up and diagnosis of reproductive problems. The aim is to characterise different types of herds with different problems of reproduction and to adapt the advice and the strategy/means to conduct further investigations to those types of herds / situations. This program is elaborated together with the National Veterinary School of Nantes (UMT Santé des Troupeaux bovins). The last part of the management issues is to communicate about reproduction, with documents and forms dedicated to AI centres. A manual describing the quality procedures in AI has been elaborated in 2003 and a guideline for AI technicians and farmers about reproduction and insemination management has been prepared.

These different tools have to be used simultaneously to touch a large number of farmers and technicians and to act in a synergic way to optimize reproduction results in dairy cows.

2.0 Genetic tools to improve fertility

Genetic selection in dairy cattle has been evolving considerably within the last years. As an example, the genetic evaluation for the Holstein breed is made in France since 2008 by using several hundreds of markers/character instead of 30 QTL/character in the previous MAS evaluation (Fritz 2010). Recent genomic tools give the possibility to breeding companies to implement Marker Assisted Selection (MAS), which allows to evaluate very young candidates with a good precision: sets of thousands of genetic markers are today available to select animals (Ducrocq 2010, Fritz 2010). The evolution of sequencing techniques as well as the manufacturing of very powerful chips (already used for human research) will probably make available the use of the complete genome information for selection purposes in a few years. Due to those technical evolutions, application to different small breeds should be the next step (Ducrocq 2010). For those breeds, one important point will be to establish a reference basis and to collect appropriate phenotypic characteristics, as they have not been so much intensively studied when compared to Holsteins (Humblot *et al.*, 2010).

Accuracy of genomic indexation has been calculated in comparison to classical selection: estimated breeding values with MAS information in very young animals (few days) are higher than those obtained at birth without MAS and after first lactation (using progeny testing). For some traits, such as fertility the precision associated to genomic indexes is much better than with classical selection (FGE, 2009; Table 1). This enough precision of MAS indexes combined with the high costs of progeny testing should contribute to reduce progressively progeny testing or even to suppress it.

Table 1. Accuracy of Estimated Breeding Value (EBV) of animals at birth, after first lactation and after usual progeny test without MAS, and of animals at birth with MAS information (FGE, 2009).

Animal age →	Sex	Birth	4-5 years	3-4 years	Few days
Trait ↓		EBV (without MAS)	EBV after progeny testing	EBV after first lactation	EBV with MAS information
Milk	Male	0.32	0.70		0.60
	Female	0.32		0.47	0.60
Morphology	Male	0.30	0.70		0.50
	Female	0.30		0.45	0.50
Female fertility	Male	0.22	0.45 (non official)		0.50
	Female	0.22		0.25 (unpublished)	0.50

In parallel with those technological evolutions consortiums are created in different countries to strengthen the value of the genomic information by including more and more animals in the evaluation and selection process (Ducrocq 2010). In autumn 2009, the EuroGenomics partners VikingGenetics (Denmark/Sweden/Finland), UNCEIA (France), DHV and vit (Germany) and CRV (Netherlands/Belgium) agreed on the exchange of genomic SNP marker data (Illumina Bovine SNP50 BeadChip). Each partner contributed the data of 4,000 proven bulls to further improve the reliability of genomic breeding values. Starting March 2010 all partners will extend their reference population to a total of 16,000 bulls. This is one of the largest reference populations in the world, but more important is its unique quality. Conventional daughter breeding values of the EuroGenomics bulls all result from sophisticated data collection systems and herdbook registration including comprehensive fertility data. Thus, data recording includes not only milk production traits (i.e. 19 million cows as daughters of the EuroGenomics bulls) but also functional traits. The new approach results in reliabilities that exceed current reliabilities of genomic proofs by about 10 %. To optimize use of genomic tools in breeding programs, recent simulations on real data tried to maximize the genetic progress, by running different scenarios of breeding schemes: generating a higher number of candidates (2400 young male calves evaluated by MAS, leading to 80 in use, instead of 800 calves leading to 130 progeny tested and 15 in use under classical selection) seems to maximize genetic progress together with a lower consanguinity (-23 % when compared to the classical evaluation; Colleau, 2010). Moreover, obtaining high number of candidates implicates to adapt the way of using biotechnologies in the breeding programs in order to optimize the genetic variability, to control the interval between generations and to lower the costs (Humblot *et al*., 2010).

These changes highlight the possibility to select animals for new traits, even with a low heritability. This strengthens the importance of the phenotypic information that must be recorded in a common way from large number of animals and the necessity to ensure the quality of the collected data.

3.0 Field to assess environmental and management factors associated with reproductive results

Field studies are very useful to investigate environmental and management factors under farm conditions. Preliminary results retrospectively issued from 309 farm audits indicated that the main sources of low fertility results are related to high energy deficit after calving and to problems around time of breeding (particularly heat detection or expression; table 2).

Table 2. Principal risk factors retrospectively identified in 309 farms audited for reproductive problems by AI and technicians from the milk control association (Ponsart et al., 2005).

Risk Factors	Number of farms	%
High energy deficit after calving	191	61.8 %
Heat detection or management of breeding	190	61.5 %
No principal factor and / or mineral deficit	111	35.9 %
Management of heifers of dried cows	105	34.0 %
Low hygiene at calving	77	24.9 %

Following this, a set of field trials were initiated in 2004 in the Holstein breed aiming to study all the factors that may influence AI results (heat detection, restraining of cows…..), during the 3 first months of lactation (regularity of cyclicity in relation to body condition score) and to study management feeding practices before and after calving. The largest trial called FERTILIA was realized in 135 Holstein dairy herds to describe at both individual and herd levels management practices for heat detection, restraining of cows and nutrition. As heat detection practices have been identified as a key point in herd management, the results will be focused on practices related to heat detection and insemination. Morever, the recent development of new automated tools may change the way of detecting estruses or spotting unhealthy animals and therefore managing reproduction within a herd. Thus, the use of automated heat detection devices has been evaluated under different field conditions.

3.1 FERTILIA: insemination practices

Individual first artificial insemination (AI) conditions were recorded from 135 herds and 4667 1st AI's by AI technicians (Fréret *et al*., 2006). Progesterone was measured in milk (ELISA) on the day of AI and 21 days later to determine the incidence of non-fertilization or early embryonic death (NF-EED), as described by Humblot (2001). Pregnancy was checked by ultrasonography between 45 and 75 days following first AI, in order to estimate the incidence of late embryonic death (LED) and conception rates following first AI. The effects of individual practices related to heat detection, calving and AI conditions were simultaneously tested with a multivariate mixed logistic model. Cows with a short calving to 1rst AI interval (< 60 d), with 3 lactations at least, with difficult calving conditions, without sexual behavioral signs or mounting activity during estrus presented lower conception rates than the other females. On the contrary, when AI was performed within 18 hours following heat detection and occurred from September to February, higher conception rates were observed (Table 3; Freret *et al.*, 2008).

The frequency of NF-EED was significantly increased when other signs of oestrus than standing heat or mounting activity (alone or in association) were used to call the AI technician. Time of AI (relative to heat detection) as well as restraint quality was associated to NF-EED. Both early (up to 6 hours) and late insemination (later than 24 hours) increased the incidence of NF-EED, but also of LED (Figure 1).

Table 3. Individual factors associated with conception rates (CR) in a multivariate mixed logistic model (Freret et al., 2008).

Variable Class (n)	Estimated CR (%)	Odds ratio
Calving to 1rst AI interval (p=0.04)		
<60 d (n=539)	37.4 *	0.79
[60-80[d (n=1079)	43.1	1
[80-100[d (n=672)	42.2	0.96
[100-180[d (n=477)	46.6	1.15
Rank of lactations (p=0.002)		
1rst (n=1126)	47.9	1
2nd (n=760)	43.8	0.85
3rd (n=425)	39.4 *	0.71
≥ 4th (n=456)	38.2 *	0.67
Calving Conditions (p<0.0001)		
Without assistance (n=1820)	50.0	1
With easy assistance (n=739)	44.8 *	0.81
With difficult assistance, caesarian section (n=208)	32.6 *	0.48
Heat Sign(s) leading to call the AI technician (p=0.01)		
Standing heat (n=1095)	42.7	0.88
Mounting (n=539)	44.4	0.95
Sniffing/Licking the vagina of other cows (n=73)	52.8	1.32
Mucus vulvar discharge/ Restlessness/Drop in production/Mooing/Looking to calendar (n=278)	32.3 *	0.56
Several signs including standing heat (n=501)	45.8	1
Several signs including mounting (n=166)	41.9	0.85
Several signs including Sniffing/Licking the vagina (n=43)	44.0	0.93
Other signs (n=72)	35.4	0.65
Interval from the heat detection (sign leading to call the AI technician) and AI (hour) (p=0.01)		
[0-6[h (n=498)	43.2 *	1.37
[6-12[h (n=586)	47.4 *	1.62
[12-18[h (n=767)	44.0 *	1.41
[18-24[h (n=526)	41.3	1.27
≥24 h (n=390)	35.7	1
Season of AI (p =0.002)		
Sept-Oct 2004 (start of the AI period) (n=553)	45.3 *	1.54
Nov-Dec 2004 (n=1074)	45.1 *	1.53
Jan-Feb 2005 (n=652)	44.0 *	1.47
March-April-May-June 2005 (n=488)	34.9	1

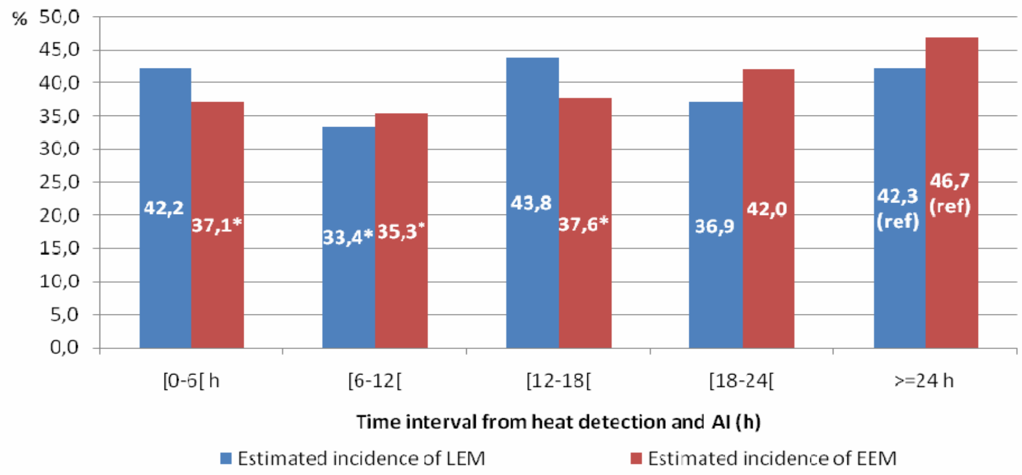

Figure 1. Effects of time interval from heat detection (sign associated with the call of AI technician and first detected sign) to insemination and embryonic mortality
*(Early = EEM, Late = EEM; * significantly different from the reference.*
From"FERTILIA", UNCEIA, Humblot et al., 2009).

3.2 FERTILIA: Restraining practices

Places and means of restraint were grouped together according to their effect on NF-EED rate and classified as UFR= unfavourable, IR= intermediate and FR= favourable type of restraint. Unfavourable restraint comprised box with corridor or cubicle without cow being tied-up or cow inseminated in the milking parlour. Intermediate conditions were those in which AI was performed in loose housing conditions or in a box, with head-locking restrainer, or box with a barrier. Favourable restraining conditions were identified as box with head-locking restrainer or corridor with restraining bar or tie stall barn or cubicle with cow being tied-up by the farmer or cow tied-up with a rope or a halter. Lack of fertilization and NF-EED rate was globally 36.8%, and was influenced by the type of restraint: UFR, 40.7% (n=398), IR, 37.1% (n=2961) and FR, 32.2% (n=488), (Figure 2) and by the quality of the restraining method as judged by the technician at time of AI: good, 34.8% (n=2188), nearly good, 38.9% (n=1675), bad/nearly bad, 37.2% (n=277) (Freret *et al.*, 2007).

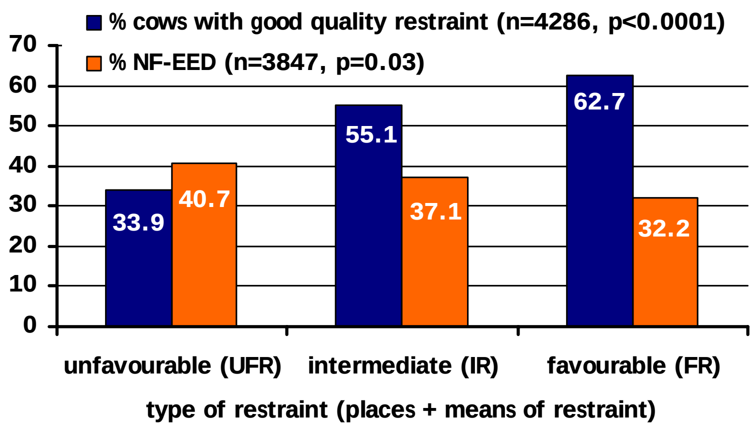

Figure 2. Percentages of cows with a good quality of restraint as judged by the AI technician and effect of the restraining conditions on the frequency of lack of fertilization and early embryonic mortality (NF-EED) (from "FERTILIA": Freret et al., 2007, Ponsart et al., 2007).

Not so much data exists in the literature on the effects of those factors related to AI conditions. It may be speculated that when conditions are unfavorable (i.e. in case of lack of clear signs of estrus, bad

restraining conditions, and bad timing of AI) these different factors may induce a lack of fertilization or fertilization at a time which is not optimum for oocyte quality and subsequent embryo development.
The time of detecting estrus during the day influenced LED. Incidence of LED was increased when detection occurred during feeding compared to a period of inactivity (48 vs 34%, p<0.05). The incidence of LED was increased also when AI was made on induced estrus when compared to natural heat (44 vs 35.5%, p<0.05) (from "FERTILIA", UNCEIA, unpublished data). Despite negative progesterone concentrations at time of AI were measured in those cows, a possible confusion with bad timing of AI and cyclic irregularities may exists for those sources of variation of LED.

4.0 Practical tools dedicated to farmers and technicians for reproductive follow up

A first part of this work aimed to develop methods for herd follow-up to diagnose reproductive problems. The strategy consists to characterise different types of herds with different reproductive problems and to give specific advises to modify management and eventually to propose additional investigations in some herds. This program is conducted in partnership with the Livestock Institute (Institut de l'Elevage, Paris) and National Veterinary School of Nantes. The last part of the management issues is to communicate about reproduction, with documents and forms dedicated to the AI centres and/or to farmers.

4.1 Practical tools in breeding management: needs and demands

The attitudes and expectations of farmers in terms of technical support or services are ranging from «self-working» farmers "picking" some information on reproduction to farmers expecting assistance from experts or farmers working regularly with AI technicians to follow-up reproductive results (Frappat *et al*. 2005; Magne 2005). Moreover, a large variability has been observed between farmers when considering the concept of « eproductive problems». This is partly explained by their different production and herd management objectives, by differences in the follow-up of reproductive performances and the conditions under which they can compensate low reproductive results (such as culling).

Several factors are combined to interpret the performances of reproduction at a herd level, which may lead to discrepancies between the needs formulated by farmers and those sensed by technicians or veterinarians. Nevertheless, AI cooperatives, technicians or veterinarians should propose a wide range of tools ranging from individual assistance to booklets explaining advantages related to the use of different practices, in order adapt their services to the different profiles of farmers and to repeat the important messages through different communication channels.

Two qualitative surveys were realized in 2004 and 2005 to investigate the needs and expectations of farmers in terms of reproductive follow-up and learning sessions. About 100 farmers, as well as technicians and veterinarians were individually interviewed for 2 hours with the help of a questionnaire about their needs in terms of reproductive management and the modalities of reproductive follow-up used in their herd. From this study it was shown that most often several sources of information were available for farmers in the field of reproduction, and in that case the different persons involved seemed to fail convincing farmers to change their farming practices.

According to Tancerel (Tancerel 2004), 46 à 91 % of 71 interviewed dairy breeders may use some risk practices in heifer management, calving intervention, heat detection and restraining cows before AI. The use of risk practices seems to be more related to an individual perception of farmers than to a general lack of knowledge in terms of "physiological" processes. This leads to promote tools based on convincing methods, with a personalized approach including psychological barriers and motivations (Dockès *et al*., 2005). Two different tools seem to correspond to this approach: an individual follow-up (with regular visits, documents and discussion) or a small training group with debates, exchanges between participants (Kling-Eveillard 1996).

As recently described by Mee (2009), technicians and veterinarians cannot simply wait for the farmer to call for this specialized service, it must be promoted. The primary route to this goal remains through opportunities created by the clinical reproductive problems encountered by clients, so-called 'contact moments'. Rather than continuing to focus on problems of the indicator cow, the veterinarian or technician needs to focus on what this cow tells us about the herd, become more 'data-literate' and realize that by repeatedly observing individuals alone they cannot influence the herd performance. Presented with these opportunities, particularly with a receptive client, it is up to the technician to attempt to lever existing relationships to get higher levels of interaction with herd owners. Receptive clients have been defined as those with larger herds, fertility problems, higher education levels, members of agricultural organizations and farm managers (Mee, 2009).

4.2 REPRO Action: Evaluation of an individual retrospective approach

As the AI cooperatives decided in 2002 to develop new tools to optimize reproductive results in dairy cows, a preliminary study was performed to evaluate some previously existing methods and tools designed to help farmers in the field of reproduction: some positive and negative aspects were pointed out by farmers, technicians and veterinarians during interviews. Three different kind of existing services have been identified: individual approach, small training groups and large plans of communication (Table 4).

When considering the individual approach, the most critical points were related to the implication of different partners (to coordinate the delivered messages), the use of a standardised method (which facilitates audit realization) and the absolute necessity to continue the assistance after a first visit (Table 4). In response to those observations, a standardised method aiming to diagnose reproductive problems "REPRO Action" was developed with a more structured approach (estimation of performances in different groups of cows, herd visit, research of potential sources of reproductive problems, plan of action discussed with farmer, reports) including a precise guideline for technicians and a computer analysis to estimate the existing and future reproductive performances. This approach is in agreement with other methods reported by Mee (2009), describing 3 successive stages of implementing veterinary herd fertility management: 1/establishing current herd fertility performance, 2/ investigating the factors associated with it and 3/ designing a program to improve it.

Table 4. Main tools proposed by the AI cooperatives and the UNCEIA team to help farmers and technicians to better manage reproduction.

Type	Tools existing before 2002 *	New tools developed
Individual approach	16 methods (4 evaluated from 88 individual interviews*) Important role of partners (technicians, veterinarians) to coordinate the technical messages. One appreciated method: from a quantitative estimation of reproduction criteria, identification of groups of cows or particular periods related to the reproductive problem. Use of a standardized method, with a real herd visit and precise documents. Necessity to implement a follow-up after the first visit to verify the effectuation of the plan and the results.	REPRO Action for dairy (2005) and beef herds (2006) Standardized method in 6 steps, leading to a plan of action and an evaluation of results. Dedicated to herds with «low» reproductive results. With a computer analysis.
Small groups	15 actions (teaching session or information via meetings) Farmers are not motivated by theoretical meeting. Practical and concrete approach is needed.	«Succeed AI together» (2007) Two days of debates/exchanges on reproduction and AI managements. Dedicated to all farmers.
Large plans of communi-cation	3 kinds of worksheets, 4 guides or books in the field of: heifers management, calving, calvind to breeding interval, semen production, heat detection, AI and restraining No exhaustive guide including all aspects	"REPRO Guide" (2005-2006) Referential on reproduction, including 37 worksheets (physiology, female and herd management, analysis of reproduction parameters, Dedicated to farmers and technicians,

* Data from 25 AI cooperatives and 88 individual interviews in 4 AI cooperatives (31 AI technicians, 26 partners and 31 farmers; (Ponsart *et al*., 2008).

A first technical evaluation has been realized in 2007, indicating that 255 herds took benefit from a first visit in 2006. From 132 herds visited between September 2005 and October 2006, the proposed actions have been retrospectively evaluated from a phone survey (with 105 farmers) and associated with their real application. A large part of the farmers (83 %) were satisfied by this individual approach and 73 % of them considered that the follow-up proposed by the AI technician was sufficient and improved reproductive performances in their herd. A majority of actions were dedicated to dairy cows (61 %). About 60 % of the planed actions have been applied in a rapid and complete manner. It is interesting to note that the most frequent recommendation that aimed to improve heat detection was not adopted in most of the cases (Ponsart *et al.*, 2008).

4.3 REPRO Pilote: Monitoring reproduction according to preset goals

Watson (2009) has emphasized the need for this to be a continuous in-going cycle rather than a straight line audit (Figure 3; reported by Mee, 2009). In order to complete the investigative audit, a scheduled monitoring of reproduction may be proposed to farmers. Therefore, REPRO Pilote has been designed as a preventive approach, aiming to monitor reproductive events according to preset goals (Table 5).

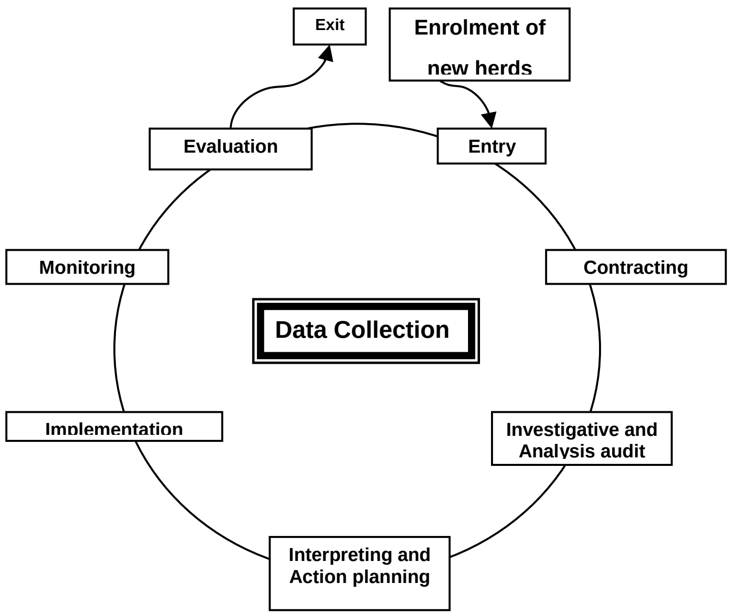

Figure 5. The bovine fertility management cycle (Watson, 2009 reported by Mee, 2009).

Table 5. Description of 2 different individual approaches of herd fertility management.

Retrospective investigation of problems	Scheduled monitoring of reproduction
Aim: identify source of problems	Set goals and assist farmers in reproduction management (according to the management strategy)
Retrospective approach	Preventive approach
Decreased performances of reproduction	All farmers
Complete visit + evaluation of plan (standardized method)	scheduled and frequent visits (standardized method)
Plan of «corrective actions»	Adapted tools to facilitate the monitoring of reproductive events: Pregnancy checks, lists of females…

When using REPRO Pilote, the ultimate goals should be set by the farmer and the role of the technician is to assist the farmer in measuring and achieving these goals. Goals should be SMART (specific, measurable, attainable, relevant and time-limited; Mee, 2009). Four different herd situations have been defined in order to help technicians to focus the monitoring on the more relevant reproductive performances according to the following "production strategies": high milk productivity, low cost of milk and work organization.

After having set personalized goals, the technician may propose the most relevant tools to facilitate the herd follow-up such as pregnancy checks, list of females or body condition scoring. Finally, a plan of actions is proposed with intermediate visits, which are scheduled according to the herd situation (production strategy, seasonality of calving). This approach is actually tested in 40 farms, from 5 different AI centres, with standardized documents and a previous training course. This may help AI centres to standardize and implement herd fertility management programs which may be compared a hazards analysis critical control point (HACCP) approach (Lean *et al.*, 2003).

4.5 REPRO Guide: a referential widely diffused to AI technicians and farmers

All summarized messages from the literature and from previous field experiments were gathered in a guide "REPRO Guide" that contains 37 illustrated worksheets on bovine reproduction. This referential describes the basics in reproductive physiology, AI management and semen production, herd management and the analysis of reproduction criteria. A total of 2090 guides have been diffused to all French AI technicians and engineers involved in reproduction. About 3300 supplementary books have been distributed to farmers, agricultural schools and other partners involved in development activities. Actually, several sets of slides have been prepared to facilitate the utilisation of the REPRO Guide during teaching sessions.

5.0 Conclusion

Several sources of information are available to quantify and qualify reproductive performances at a national level (genetic indexes, detection of genetic markers) and at the herd level (analysis of reproductive performances). The combined use of genetic and practical tools may improve reproductive results in the next years.

At the herd level, it is necessary to convince breeders to modify some of their practices to decrease the impact of detrimental environmental factors such as bad restraining, and bad management of the peri-partum period. When based on precise estimation of the reproductive performances, field trials help to actualize and precise the practices to be recommended under field conditions. Such trials also facilitate the appropriation of the results by breeders and this contributes to increase the application rate. However, some domains remain problematic. Most particularly, heat detection and expression remain one of the most important problems in dairy herds, mainly because in case of bad results, it is difficult to separate properly the detection and expression characteristics within a herd and also because, when formulated, the recommendations are not often applied. New methods of detection (such as automatic methods) and/or new management strategies (in relation with milk production), which could involve economical criteria should be developed to improve heat detection.

Finally, sanitary aspects and the way pathogens interact with reproductive events should be probably more precisely investigated. Additional tools and alternative strategies may be developed especially in the herds were the use of above described tools were not efficient enough to improve reproductive performance despite a good application of recommended measures.

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