European Cooperation in Science and Technology - COST -

Secretariat -------

Brussels, 4 July 2012

COST 4126/12

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action FA1201: Epigenetics and Periconception Environment - Periconception environment as an epigenomic lever for optimising food production and health in livestock

Delegations will find attached the Memorandum of Understanding for COST Action as approved by the COST Committee of Senior Officials (CSO) at its 185th meeting on 6 June 2012.

MEMORANDUM OF UNDERSTANDING
For the implementation of a European Concerted Research Action designated as

COST Action FA1201
EPIGENETICS AND PERICONCEPTION ENVIRONMENT PERICONCEPTION ENVIRONMENT AS AN EPIGENOMIC LEVER FOR OPTIMISING FOOD PRODUCTION AND HEALTH IN LIVESTOCK

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 4154/11 "Rules and Procedures for Implementing COST Actions", or in any new document amending or replacing it, the contents of which the Parties are fully aware of.

2. The main objective of the Action is to establish a network of European researchers and industries working together in order to define how the periconception environment influences the production of healthy, fertile and productive livestock and which factors provoke epigenetic changes in gametes/embryos.

3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 80 million in 2012 prices.

4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.

5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter V of the document referred to in Point 1 above.

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A. ABSTRACT AND KEYWORDS

Parental stress before, during and after conception (i.e. the periconception period) induces epigenetic changes in gametes and embryos. Such epigenetic changes may adversely affect the future health, development, productivity and fertility of those offspring. While there is increasing evidence for this in agricultural species, most of this knowledge derives from epidemiological studies in humans and controlled studies in laboratory animals. In this COST Action, time frames and mechanisms during which the gametes and early embryos are susceptible to epigenetic modifications will be determined in livestock in order to optimise their health and productivity. This COST Action will identify stressors and molecules which induce, modulate or remove epigenetic marks on genes that are relevant for different applications in farm animals. Public engagement activities are planned during the COST Action to inform the general public on the importance of the epigenome via the periconception environment in future food production, health and welfare. Research on epigenetic control of development is being performed by different groups in the EU, but efforts need to be coordinated in order to avoid duplication, set targets and guidance for future research and to standardise protocols in this field through a large collaborative EU network. These goals can only be achieved under a COST programme.

Keywords: epigenetics, gametogenesis, embryogenesis, livestock, periconception environment

B. BACKGROUND

B.1 General background

It has become increasingly evident in recent years that the environment during the earliest stages of development has a direct and profound influence on the health and future developmental capacity of the individual. Whereas the genotype is determined at fertilisation, there exists a period during this early stage development, when phenotypic expression of the organisms genotype is adjusted to match environmental cues. These adjustments in gene expression are established "on top" (i.e. “epi” in Greek) of the genes, and this area of research is therefore referred to as epigenetics.

Understanding the epigenetic mechanisms involved in embryonic development will help to address such issues as (i) the risks associated with stress, illness or dietary restrictions and metabolic imbalances during the peri-conceptional period, (ii) the effects of maternal and paternal nutritional status/stress on epigenetic programming through the germline, and (iii) transgenerational studies where, in future, greater emphasis in livestock species should be placed on traits of agricultural importance.

With the introduction of new techniques such as Next Generation Sequencing it has become feasible to perform high-throughput screening to analyse environmental effects on samples containing a limited number of cells, e.g. embryos or gametes. It is therefore very timely to launch a COST Action now on this ‘hot’ topic, which will allow consolidated strategic planning by European scientists for pinpointing vulnerable periods during gamete and embryo development of livestock for these epigenetic influences. Specialised Working Groups (WGs) will set up an organised framework to avoid research duplication and to ensure “best partner” collaborations. This COST Action will develop tools to analyse epigenomic signatures, will try to link the periconception environment with adult phenotypes, and will inform the public on how “nature and nurture” can work together, to manipulate the health and welfare of livestock in a positive way, without tinkering with the genes but by inducing epigenetic changes under controlled conditions or by defining preventive measures. As a complement to this, a better understanding of epigenetic changes and regulation at the beginning of life will help to evaluate the effects of various factors on phenotypic performance for production and functional traits of interest. Such information is particularly critical in the context of genomic selection to adjust genetic indexes issued from genomics. These goals can be achieved under a COST Action programme. No other parallel project exists at present.

B.2 Current state of knowledge

At present Europe is at the forefront for this type of research; being years ahead of the USA, Canada and Japan. Scientists became aware of the fact that preconception environment can influence postnatal phenotype after reports on congenital abnormalities in calves born after *in vitro* culture of ruminant embryos (Schmidt et al., 1996; Van Wagtendonk –de Leeuw et al., 1998).

One of the most obvious traits of the abnormal offspring following use of assisted reproductive technologies (ART) was an overgrowth phenotype, and thus the syndrome was termed “Large Offspring Syndrome” (for review see Young et al., 1998). European groups reported that some of the observed foetal abnormalities, such as enlarged umbilical cord and abdominal ascites, were probably consequences of placental dysfunction (Constant et al., 2006). British groups showed that the severity of the syndrome was influenced by culture conditions (serum-containing; co-culture) (Sinclair et al., 1999; Rooke et al., 2007), animal species (ruminants) (Grace and Sinclair, 2009) and levels of maternal nutrients (high urea levels) during pregnancy (Powell et al.; 2006). The possible mechanism was related to epigenetic changes with DNA-methylation of imprinted genes that were imposed upon the embryo by its exposure to a perturbing environment during a critical period (Young et al., 2001).

In parallel research, European scientists were the first to demonstrate that maternal nutrition specifically during the peri-conceptional period has a lifetime effect on health. In sheep, diets deficient in specific micronutrients (i.e. B vitamins such as B12 and folate, and amino acids such as methionine) fed at the time of conception led to adult offspring that were insulin resistant and hypertensive, and this was associated with global alterations to DNA methylation (Sinclair et al., 2007). In rodents, a protein-restricted diet for just 3-4 days from fertilisation to blastocyst stage (i.e. before implantation) was sufficient to alter growth and induce adult hypertension and abnormal behaviour (Watkins et al, 2008). Important in-depth studies on the possible causes of Large Offspring Syndrome were carried out by Europeans, such as the Dutch (Van Wagtendonk-de Leeuw (2000) and the Italians (Lazzari et al., 2002) and more recently the French, Spanish and British groups working on the effect of nutrition, disease and peri-conception environment in rabbit, rodent and ruminant models, respectively (Picone et al., 2011; Jammes et al., 2011; Watkins et al., 2008, Banrezes et al., 2011; Evans et al., 2010; Calle et al., 2011). However, data on many more animal models need to be generated, such as rabbits, pigs, horses, and even poultry and fish.

As for now, many conferences on these topics have been restricted to just specific species (mostly rodents) and so an integrated understanding across species is now required.

It has been clearly demonstrated that assisted reproductive technologies, such as in-vitro fertilisation and cloning by somatic cell nuclear transfer, have a profound effect on DNA methylation, which is one of the major components of the epigenetic landscape (Deshmukh et al., 2011). However, there is a real gap in the current knowledge on the effect of environment and epigenetic reprogramming during gametogenesis. Especially the precise timing of *de novo* DNA methylation (a major mechanism in epigenetics) during gametogenesis is still poorly understood and is mainly limited to data in the mouse (Ooi et al., 2007). Moreover, the timing of this methylation differs between the male and female germlines, but only few data on epigenetic changes during this sensitive time frame have been reported so far in livestock

A new approach to this problem is promised by this COST Action. By reporting annually on the importance of the periconception environment at large, the COST Action will encourage:

(1) Definitive research on the effects of stressors during gametogenesis and embryogenesis, reporting on the resulting phenotype and the epigenome

(2) Follow-up on offspring conceived in different animal models exposed to stressors under specific timeframes during gametogenesis and embryogenesis

(3) Development of new methods to intervene in the cell metabolism in real time and the application of novel techniques such as next generation sequencing and live-cell imaging

(4) Driving epigenetic strategies and protocols for enhanced animal welfare, fertility and food production

(5) Communication with the general public on the importance of a healthy diet around the time of conception; reporting to husbandry groups on nutrition effects on animal production

This COST Action will be the first to work across species boundaries to relate peri-conceptional nutrition and environment to successful and improved animal production using natural epigenetic strategies, avoiding any direct genetic modification, to answer global concerns on food production.

B.3 Reasons for the Action

The old wisdom "You are what you eat" has lately not only been proven to be true but it can also be extended to "You are also what your mother and father ate". This saying directly refers to the fact that the periconception environment in its broadest sense directs the health and characteristics of a given individual. The developmental plasticity of gametes and embryos is a property which allows them to adapt and to survive. As such, it is essentially a good thing and has been used through evolution to help individuals enhance their competitiveness. Likewise, stress, infection and deficiencies in nutrition or metabolic support, which may be present at conception, can interfere with optimal development of offspring and their health during adulthood. For example, a new model developed within our consortium shows that maternal sickness (bacterial infection) at the time of conception is sufficient to alter the innate immune system in the next generation (Williams et al., 2011).

In this COST Action, early developmental steps will be mastered *in-vitro* where the environment is under full experimental control. This unique opportunity makes it possible to vary at will the flux of metabolites and signaling events from the environment and drive the internal developmental processes in real time, at the scale of a second, and thus reveal how the system as a whole responds to precise input and produces specific phenotypic traits at adult ages (Banrezes et al., 2011; Maalouf et al., 2009).

Current EU research at the level of epigenetics is either focused on human health (immunity and cancer) or on plants. To the best of our knowledge there are no EU programmes focused on animal health and production as driven by epigenetics. There are however many national research projects being funded at present on this topic, with many papers about to be published. In the coming years, a lot of important scientific data will be published which will need to be communicated to end users in the field, such as the artificial insemination industry, meat and milk producers and industries involved in poultry and aquaculture production.

The COST Action programme will establish a network between different research groups, industry, farmers and the general public (consumers), and this will facilitate communication and transfer of data between the different groups scattered across Europe. It will allow breeding companies to employ the scientific findings so the genomic selection can be managed in a more sustainable way. It will allow for mutual discussion and decision making for future strategies related to the different stages of research that need to be accomplished.

The advantages of carrying out this project within a COST programme are numerous:

- Avoiding duplication of research in different European member states
- Optimising communication between scientists, farmers, industry and consumers by internet applications as a permanent tool for dissemination, interaction and translation of new findings
- Organising informative sessions during conferences in which translational research is communicated to the industry and to farmers
- Prioritising research in areas of importance – encouraging submission of research grants to national governments
- Sharing new technologies and tools between different groups of this COST Action via Short Term Scientific Missions and Training schools for young students
- Collaborating with industry to focus on sensitive periods during gametogenesis/embryogenesis
- Mobilising pharmaceutical industries and advanced micro-engineering industries in order to intervene into the molecular pathways at the origin of the epigenetic" memories"

B.4 Complementarity with other research programmes

No other current or planned European research programme exists that has the same aims and goals as this COST Action programme. However, this COST Action has links and complementary ties with a number of funded projects.

- **PLURISYS (**Funded by FP7). Systems biology approaches to understand cell pluripotenc**y**

- **EPIHEALTH** (Funded by FP7). Integrated approach to understand mammalian & human ageing
- **EPIGENESYS** (Funded by FP7). Network of Excellence for epigenetics and systems biology.
- **FA1002** (COST Action) Farm Animal proteomics.
- **ANGIOPLACE** (European Research Council). Placental angiogenesis and vasculogenesis.
- **BRIGHTANIMAL** (Funded by FP7) Multidisciplinary approach to practical and acceptable precision livestock farming for SMEs in Europe and world-wide
- **RGB-NET** (Funded by FP7) COST Action Rabbit Genome Biology (TD 1101)

C. OBJECTIVES AND BENEFITS

C.1 Aim

This COST Action will define how the periconception environment influences the production of healthy, fertile and productive livestock and which factors provoke epigenetic changes in gametes/embryos. Specific expected deliverables; -Definition of the vulnerable _epigenetic_ timeframe for gametes and embryos of livestock, poultry and aquatic species -A list of molecules/nutrients/vitamins/pharmaceuticals that can induce epigenetic changes during this timeframe -Providing information to industry, breeders and general public about the impact of the periconception environment on animal production, reproduction and welfare -The development of new methods for controlling the functioning of gametes and embryos by designing *in vitro* culture conditions with the involvement of industry -Definition of preventive measures to be applied before fertilisation and during conceptus development to avoid unfavourable epigenetic effects with conventional breeding methods. -Definition of similar measures and improved protocols when using assisted reproduction -Definition of potential epigenetic effects by pathogens on reproductive tissues and embryos

C.2 Objectives

The main objective of this proposal is to bring researchers from different European countries together, who are currently working on the epigenomic signature in gametes and embryos of different livestock species. This COST Action will answer important questions in the field, in order to:

I) Develop an epigenomic toolbox including next generation sequencing technologies and bioinformatics: due to rapid changes in molecular tools these techniques will allow for large scale screening of epigenetic changes in gametes and embryos.

II) Define the factors that can influence the epigenetic profile during the periconceptional period : these factors can be physical stressors like cold or heat, high pressure, nutritional status of the animal, maternal health or sickness, feed supplements or deficiencies, pharmaceuticals, endocrine factors, culture media

III) Define the timeframe at which most epigenetic changes take place: spermatogenesis takes 2 months in most livestock; oocyte and follicular development takes 3-4 months; fertilisation that remodels parental chromatin takes a few hours; and embryogenesis, including development of the germline (the precursors of sperm and eggs) which undergoes epigenetic reprogramming during the first months of pregnancy.

Each of these developmental periods will be addressed.

IV) Define the range of optimal periconception environments that ensure healthy offspring : based on experimental data a definition will be made for each species studied.

V) Compare the susceptibility of different species (livestock, poultry, fish) and different model systems (*in vivo* vs *in vitro*) to epigenetic disturbances

VI) Interact with stakeholders and the artificial insemination industry to discuss the extent to which information generated can be integrated in the implementation of genetic schemes and in the adaptation of gamete and embryo production protocols (i.e. semen collection, cryopreservation of semen and embryos, superovulation, IVF…)

VII) Inform the scientific community and the public , especially people involved in animal production, about altering the epigenome via the periconception environment.

Achieving these objectives will benefit livestock production as well as public health in Europe.

C.3 How networking within the Action will yield the objectives?

These objectives will be achieved by the organisation of Annual Conferences, workshops and technology visits between European research labs. The COST Action will develop an advisory brochure on how to treat breeding animals in order to produce healthy offspring, including a better adaptation to the environmental changes that are occurring at present.

C.4 Potential impact of the Action

Understanding the impact of environmental changes on gametes and embryos is of major scientific, health and economic value. Some of the benefits are;

-Manipulation of qualitative and quantitative traits by epigenetics

It has been shown that it is possible to manipulate maternal behaviour, muscle growth and mammary gland development by epigenetic mechanisms (Jammes et al., 2011). These are important traits for animal breeding and livestock production. It is noteworthy that by understanding epigenetic mechanisms and the molecular pathways that activate them, exceptional progress can be achieved in animal breeding by natural means and /or defining preventive measures.

-Improvement of livestock health by epigenetics

Epidemiological studies in human and rodents have shown that the origin of diseases such as coronary heart disease and type 2 diabetes lies within the periconception environment. Factors orienting the developmental process and affecting the developing foetus may compromise health in adulthood. Although less well documented than metabolic diseases, exposure of reproductive tissues to pathogens has also been reported to induce unfavourable effects in relation to epigenetic mechanisms in the human. Hence, understanding the periconception environment and its effect on the epigenetic profile of an individual can help improve livestock health.

-Promoting livestock fertility, resulting in the birth of normal offspring

Fertility of high producing dairy cattle is declining with less than 35 % of inseminated dairy cattle conceiving per cycle, due to genetic selection solely orientated towards milk production. Today, due to better balance in the weight given to multiple traits (including fertility), reproductive performance is on the way to stabilisation or even improvement (Humblot, 2010). The precise understanding of the functions of epigenetics is even more important in the new context of genomic selection, where animals will be selected on DNA characteristics and not from a phenotype integrating possible epigenetic effects. This COST Action will improve selection procedures while adjusting data from genomics by data generated from knowledge on epigenetics.

C.5 Target groups/end users

Beneficiaries of the results of this COST Action are:

- **Researchers working in the field:** Never before has such a multispecies consortium been assembled at a European level to address issues described in this COST Action. Many researchers working with different species have been contacted and expressed their interest.
- **National government policy makers, European level policy makers:** Necessary to direct future funding calls that will provide the grants needed for basic research.

- **Livestock breeders, poultry breeders, aquaculturists:** The interest of such stakeholders for more knowledge in epigenetics is obvious from their strategic agenda for research in which this topic is cited as one as the strongest priorities
- **Food industry, artificial insemination industry and clinicians:** They need to understand the relationship between maternal health and nutrition at conception and the disease risk for the next generation. Semen producers such as CRV have expressed interest in the results and will attend the meetings.
- **Biotechnology industries**: Pharmaceutical companies, microengineers and nanotechnologists have been contacted and have expressed interest in participating in this COST Action. (e.g. Elvesys (F))
- **Environment and biodiversity:** In a broader sense this COST Action also refers to "slow food". The distinct taste of meat reflects the robustness of a breed created by natural selection, resulting in resistant animals that are adapted to harsh natural conditions
- **Public health, consumers, public opinion:** Dissemination of knowledge of this COST Action will lead the public opinion and consumers to a better understanding and acceptance of Biotechnology, as a discipline with potential to introduce changes for a better way of life, as Epigenetics can successfully compare with genetic modifications in obtaining similar results using more friendly and sustainable mechanisms.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

The overall goal of this COST Action (EPICONCEPT) will be to address how the periconception environment influences the production of healthy, fertile and productive livestock; and to identify which dietary and environmental factors lead to epigenetic changes in gametes and embryos. In order to achieve this goal, different questions need to be answered. These are at present being performed in nationally funded and ongoing research projects dispersed all around Europe.

1. Epigenomic tools

What is epigenetics?

Epigenetics is the study of heritable changes in gene expression and/or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence. Well-studied examples of epigenetic modifications are DNA methylation and covalent modifications to associated histone proteins, both of which serve to alter gene expression without altering the DNA sequence of genes. Epigenetic modifications occur to DNA during the normal course of development and serve to define differentiation and tissue-specific gene expression. A central emerging concept proposes that there is an 'epigenetic code', which considerably extends the information potential of the genetic code. Thus, one genome can generate many 'epigenomes' as the zygote progresses through development and translates its information into multiple cell types.

Each change in the environment of the developing embryo will affect either DNA methylation of genes or can modify histones and subsequently silence expression of particular genes, to register, signal or perpetuate altered activity states (Bird, 2007). Hence the epigenome can be changed by environmental influences, and epigenetic modifications can be mitotically inherited. Heritability of such an environmentally altered epigenome to following generations will also depend on the ability of these alterations to escape germ line reprogramming during meiosis (Dunn et al., 2010). In the life of a mammal, there are at least two critical periods in which epigenetic reprogramming occurs: the first during sex differentiation and the formation of the gametes (Kobayashi et al., 2012); and the second during preimplantation embryo development (Reik et al., 2001; Tomizawa et al., 2011). Particularly during and following syngamy, and at the blastocyst stage, which includes the first differentiation event into the inner cell mass or the trophectoderm, a dramatic increase in DNA and histone methylation takes place (in the blastocyst predominantly in the inner cell mass) (Reik et al., 2003). After implantation, further environmentally-induced epigenetic modifications can occur resulting in modified adult phenotypes (Lillycrop, 2011; Burdge et al., 2007).

How can epigenetics be studied?

One approach to study epigenetics is to focus on molecular techniques which can be used to analyse epigenetic changes. Methods for assessing DNA methylation and histone modifications at both the global and the gene level will be explored. Epigenetic information is also encoded in proteins, so proteomic approaches are useful for molecular epigenetics too.

A second approach is to use different imaging methods. For documentation of the influence of epigenetics on gamete-maternal interaction and embryo-maternal communication, innovative live cell imaging techniques can be used, i.e. digital videomicroscopy, confocal endoscopy or optical coherence tomography(OCT), or "*ex-vivo*" imaging of various epigenetic modifications in living embryos for several cell-cycles (time-lapse microscopy).

A third approach is to intervene with epigenetic marks (without using transgenics) by using e.g. molecules that interfere with epigenetic changes conferred to the DNA, such as Histone Deacetylase inhibitors. Current front-runner candidates for new drug targets are Histone Lysine Methyltransferases and Protein Arginine Methyltransferases.

To conclude, in order to develop an epigenomic toolbox, emerging new technologies such as visualization of epigenetic modifications in living cells, next generation sequencing, high resolution proteomics and bioinformatics will be adopted by the COST Action. Short Term Scientific Missions and Training Schools will be held to instruct people how to apply these technologies.

2. Periconception environment

The periconception environment encompasses ontogenesis and the organs and tissues in which gametogenesis, embryogenesis, implantation and placentation take place. Although sexual reproduction is globally robust, it is also a vulnerable process. Gametes and embryos are especially vulnerable to epigenetic changes. Some epigenetic imprints are naturally erased in the preimplantation embryo and in the primordial germ cells in order to down-regulate the inheritance of epigenetic (acquired) information between generations, and appear again later on.

Likewise, epigenetic processes are responsible for laying down the gender-specific imprinting that allows for gender-specific gene expression of paramount importance for embryonic development and placentation. As such, environmental changes during this susceptible period may affect the dynamic reprogramming process. In livestock it is not clear which stages of gametogenesis and embryogenesis are most susceptible. Such studies are already ongoing but not all technical expertise is present in every lab. Short Term Scientific Missions will link and bring together the necessary expertise to obtain these results.

Epigenetic changes may be less harmful than genetic mutations since they are reversible. However, it is most important to understand which are the healthy settings for the periconception milieu in order to avoid deleterious epigenetic changes. This knowledge will facilitate the potential to change this environment to attain the ideal conditions to which breeding animals and embryos should be exposed in order to prevent the occurrence of epigenetic mutations .

The periconception environment can be divided into:

Preconception: the environment to which growing gametes are exposed since important histone modifications take place during spermatogenesis, but also during the period of where ovaries may undergo controlled stimulation or the period of follicular growth or retrieval of immature oocytes for assisted reproduction.

Conception: Fertilisation is the process during which sperm and oocyte unite and initiate the dynamics of the developmental programme, which produces all the cell types of the ultimate adult organism. In internal fertilisers, the female reproductive tract is the venue of fertilisation. In some livestock species, such as cattle, fertilisation can also be performed *in vitro* for breeding purposes

Postconception alluding to the period of embryonic and foetal development, which includes organogenesis and development of the offspring germline (the precursors of sperm and eggs) undergo epigenetic reprogramming during the first months of pregnancy

It is now accepted that the "periconception environment" is a dynamically evolving environment exerting a combinatory effect on the future phenotype. This milieu progresses and adapts in response to sex hormones, gametes and embryos. It has recently been demonstrated that bull spermatozoa showed a decrease in chromatin protamination when exposed to heat stress during epididymal transit and during spermiogenesis (Rahman et al., 2011). It appears from very recent research that bovine imprinted gene methylation is increased in differentially methylated regions during oocyte growth in cattle (O' Doherty et al., 2011). During fertilization, enzymatic actions on the nuclear NAD levels and Sirt1 can regulate body weight in mice (Banrezes et al., 2011). Data from animal studies have clearly shown that IVF, if it includes embryo culture in certain media, is associated with changes in epigenetic marks (Fauque et al., 2007) and the expression of imprinted genes which can result in "large offspring syndrome" (Hiendleder et al., 2006; for review see Young et al., 1998). Multiple factors such as the mother's health and nutritional status as well as the presence of infectious agents, all contribute to creating a wide range of conditions affecting the periconceptional environment. EPICONCEPT refers to a situation in which the present and future of the offspring are being dictated by the past.

There is a need to coordinate the efforts of different groups on different aspects of periconception environment. Published data are at present not efficiently used to obtain a coherent picture of possible influences of the periconception environment on livestock development and offspring. Defining the vulnerable timeframes for epigenetic changes during development can only be achieved by a bottom-up approach, in which different tools, model systems and animal species, are brought together in order to get an overview of the different levels at which epigenetics can play a role in future livestock health, fertility and production.

3. Cross-species epigenetics, gametogenesis and embryogenesis

Several animal models will be used to study epigenetic effects on developmental outcome. In this COST Action, rapidly developing and short generation time rodent models will be used to define epigenetic mechanisms and strategies to translate to food production species. These rodent models are crucial to follow transgenerational effects and effects on consciousness and learning ability, which is much more difficult to achieve in livestock species. The livestock models will represent a major source of information, since many of the research groups involved in this COST Action have ongoing research projects on pigs, sheep, cattle, rabbits or horses. It should be emphasised that epigenetic reprogramming and its regulation diverge between species and such comparative studies are rare but very innovative. Scientists dealing with aquatic species such as fish and shrimps have the advantage that they can grow very large numbers of larvae and evaluate environmental effects. Finally, poultry species have the advantage that embryonic development can be manipulated and evaluated from egg to hatching. Several research teams have used the chicken as a model species for years for studying epigenetic effects.

In this COST Action thematic workshops will be organised to gather data on epigenetic influences on gametogenesis and embryogenesis and those will be presented across species. In mammals, embryo technologies such as embryo transfers will be used to specifically study the role of the periconceptional environment on the adult offspring phenotype. This multispecies approach is very innovative and will lead to new insights and “cross fertilisation“

4. Public, peri-conception and epigenome

In a popular language it will be explained how “nature and nurture” can go together, and how the health and welfare of livestock can be manipulated in a positive way, without tinkering with the genes but by inducing epigenetic changes under controlled conditions. Working and generating knowledge on this topic has been presented as one of the most important actions for the 5 coming years by the FABRE TP Group (Farm Animal Breeding and Reproduction Technology Platform). This shows that these ideas are commonly shared by research and industry, but that it is very important to gain a larger public support for its future development.

D.2 Scientific work plan methods and means

The scientific goals of this COST-Action will be achieved by organizing an Annual Conference, in which both advanced and young researchers will be able to present their research, and by educating young scientists by means of regular Workshops and Training Schools. Publications of key note lectures from Conferences or Workshops will be prepared by means of special issues or research fronts for scientific journals such as Theriogenology or Reproduction, Fertility and Development. Exchange modalities between laboratories will be possible by Short-Term Scientific Missions. Communication with industry and with the general public will be ensured by means of a special EPICONCEPT-website dedicated to translation of the results in lay terms, and by sending out regular newsletters to companies which are in one way or the other connected with this type of research, such as pharmaceutical companies and artificial insemination companies.

The COST Action will be organised by 4 major Working Groups (WGs): WG1 - Epigenomic tools; WG2- Periconception environment; WG3- Cross-species epigenetics, gametogenesis and embryogenesis; WG4- Public, peri-conception and epigenome

All WGs will contribute to the production of a book in the final year of the COST Action for further dissemination of the Action achievements

WG1 : Epigenomic tools

The objective of this WG is objective (I) mentioned in section C.2.

This objective will be achieved via following deliverables:

a) To organise focused Workshops called “Epigenomic toolbox”, one at year 1 focusing on Next Generation Sequencing and one at year 3; focusing on techniques to analyse proteins (proteomics).

b) To organise focused Workshops called “Epigenetic dynamics and highlights”, one at year 2 on the dynamics of epigenetic modifications in living cells, gametes and embryos by means of fluorescence labeling and time-lapse microscopy, and one at year 4, to instruct on the determination of DNA methylation by means of enrichment for sequences with epigenetic modifications

c) To deliver a technical publication on how to demonstrate epigenetic changes in samples with low cell numbers, derived from the four workshops.

d) To organise an inter-disciplinary Training School on “Epigenomic tools” in year 3.

WG2 : Periconception environment

The objectives of this WG are objective (II) to (IV) mentioned in section C.2.

These objectives will be achieved via following deliverables:

a) To organise a focused section, called “Epigenetic modulators” during the Annual Conference, at year 1, that will focus on factors influencing the epigenetic profile at periconception

b) To deliver a document summarizing the most important epigenetic modulators during periconception, resulting from the Annual Conference at year 1.

c) To organise a focused section, called “Epigenetic time frames” during the Annual Conference, at year 2, that will focus on developmental periods during which the epigenetic profile can be altered.

d) To deliver a document summarizing the most important periods during periconception, resulting from the Annual Conference of year 2.

e) To organise a focused section, called “Epigenetic disruptors ” during the Annual Conference, at year 3, that will focus on molecules or modulators which can confer epigenetic changes to DNA

f) To deliver a document summarising the most important epigenetic disruptors and how they can be applied during periconception, resulting from the Annual Conference of year 3.

g) To organise a focused section, called “EpiConcept ” during the Annual Conference, at year 4, that will focus on defining the right periconception environment to ensure healthy offspring.

h) To deliver a document summarizing the range of the right periconception environments

WG 3: Cross-species epigenetics, gametogenesis and embryogenesis

The objective of this WG is objective (V) mentioned in section C.2.

This objective will be achieved via following deliverables:

a) To organise an interdisciplinary Workshop on "Epigenetics in vivo and in vitro – what is the difference?", at year 1, that will focus on how to translate data from a model to the field
b) To organise an interdisciplinary Workshop on "Epigenetics and nutrition", at year 2, that will focus on how nutritional guidelines can be used to drive epigenesis.
c) To organise an interdisciplinary Workshop on "Epigenetics and stress or disease", at year 3, that will focus on how stress /pathology during gametogenesis and pregnancy can affect offspring health
d) To organise an interdisciplinary Workshop, called "Epigenetics in fish, poultry and livestock" during the Annual Conference, at year 4, that will deliver a document summarizing a comparative overview of epigenetic influences in different species.

WG4 : Public, peri-conception and epigenome

The objectives of this WG are objectives (VI) and (VII) mentioned in section C.2.

These objectives will be achieved via following deliverables:

a) To interact with the industry at the Annual Conference in a specialised practitioner's forum to integrate the generated information in gamete and embryo production protocols and in animal breeding schemes, at year 1, 2, 3 and 4.
b) To deliver a document based on these fora, to be put on the website.
c) To inform the scientific community and the general public, especially people involved in animal production, by establishing a website explaining the obtained achievements in lay terms.

E. ORGANISATION

E.1 Coordination and organisation

This COST Action is a means of coordinating several research projects in different laboratories in different COST countries that are already financed by their respective governments. Via this COST Action, efforts will be coordinated to understand how the periconception environment can affect future livestock health, fertility and productivity. The coordination of research and exchange of data between these laboratories in different European countries is achieved by realising the common milestones/deliverables of different WGs as described in section D2.

The organisation of this Action will follow rules and regulations set by COST guidelines with some Action specific variations. As described in the COST rules, the management committee (MC) will be convened by representatives of signatory countries. MC will elect a chair and a vice chair by majority vote. The MC will be divided to four Working Groups (WG 1 to 4). MC will appoint a leader and deputy leader for each WG. WG leaders are responsible for achieving goals and milestones of each WG and reporting their achievements to the MC.

After the initial MC meeting to set different WGs, the MC will meet another time during the Annual Conference later in the first year. Thereafter the MC will appoint an Executive Committee that consists of MC Chair, MC Vice Chair, Leaders and Vice Leaders of WGs. The Executive Committee will verify and approve all operational and day to day decisions and will help the Chair in smooth running the action. They will be contacted and liaised from time to time by e-mail or teleconferencing. The MC will meet once per year, preferably during the Annual Conference. During MC meetings different WGs will also meet and discuss the progress and coordination of their specific WG objectives. WG leaders will report the progress of their respective WG based on the predefined timetable to MC.

In the first MC meeting the location of the first Annual Conference will be decided. The MC will elect a conference organising committee (COC) each year that consists of the local organiser and representatives of each WG. The COC is responsible for organisation of the Annual Conference. The local organiser chairs the COC and reports its progress to the MC. Different WGs through their representatives in COC will contribute to setting the scientific programme of the annual conference. COCs are convened for one year and in each Annual Conference the location and COC of the next conference will be decided by the MC.

A dedicated website will be established to inform about the COST Action programme. as well as its progress and achievement of its objectives. Special attention will be paid to making this website informative and attractive to the lay public, to the breeding and food producing industry and to governmental organizations. It is the responsibility of the WG 4 and of the MC chair to organise the website and keep its contents up-to-date with the progress of the Action programme. In addition, dedicated mailing lists will be established as a forum to keep different MC members informed regarding the progress of the COST Action and as a mean of communication between different members of MC and WGs. Newsletters will be emailed to industrial and agricultural parties involved. If necessary, decisions regarding approval of STSMs or other matters related to this COST Action programme will be made through votes delivered via these mailing lists.

Dependent on the budgetary possibilities a number of STSMs will be financed each year for exchange of technologies and training between labs in different member states as defined in the rules of COST. In selection process for recipients of these awards, priority will be given to the younger members to take advantage of these STSMs. The application for the STSMs will be sent to the MC chair. These will be approved by the MC chair after consideration of COST rules and procedures and consultation with assessing panel convened by MC based on guidelines published by COST office. The assessing panel is chaired by the MC chair and consists of representatives of each WG.

An inter-disciplinary “Epigenomic tools” Training School will be organised by the WG 1. MC Chair and members will help WG 1 in organisation of this training school and will follow COST rules and procedures for its organisation and selection of the participants. Priority will be given to early stage researchers in taking part in the training school.

A book will be published in the final year of the COST Action detailing the achievements of this programme on understanding how periconception environment can affect future livestock health, fertility and productivity. Different chapters of the book will consist of documents produced by WGs (as mentioned in deliverables of each WG in section D2) and selected articles solicited from participants of special workshops and contributors to the general conferences. The MC Chair will be the principal editor of the book and WG leaders will constitute the editorial board for the book.

E.2 Working Groups

The core of this COST Action will be four main WGs:

- WG1 : Epigenomic tools
- WG2 : Periconception environment
- WG3 : Cross-species epigenetics, gametogenesis and embryogenesis
- WG4 : Public, peri-conception and epigenome

MC members based on their speciality and interest will take part in different WGs. Each WG will elect a leader and a deputy leader to guide the scientific progress in their respective Working Group. Leaders will report progress on WG objectives and deliverables to MC.

E.3 Liaison and interaction with other research programmes

Many experts that expressed their interest in the present Action are involved in the PLURISYS FP7 programme as well as EPIGENESYS FP7 programme that started last year. Such involvement will allow further cross-communication between programmes. In addition a number of COST Actions already exist that can be points of interaction such as “Larvae-net” and “Proteomics in animals”. Once the EPICONCEPT Action is up and running, other Actions will be contacted to obtain cross disciplinary and inter-disciplinary interaction between different programmes easily.

E.4 Gender balance and involvement of early-stage researchers

This COST Action will respect an appropriate gender balance in all its activities and the Management Committee will place this as a standard item on all its MC agendas. The Action will also be committed to considerably involve Early-Stage Researchers. This item will also be placed as a standard item on all MC agendas. During the preparation of the COST Action every effort has been made to enforce these points by consulting a gender balanced mix group of scientist and Early Stage Researchers. Especially Early Stage Researchers will be invited to apply for STSMs and to attend the Workshops and the Training Schools.

F. TIMETABLE

Timeline	Milestone
Year 1 Month 1-3	Organization of first MC meeting Establishment of the Action website
Year 1 Month 4-6	Approving first round of STSM applications year 1
Year 1 Month 7-9	Organization of second MC/WG meeting during annual meeting Achieving WG objectives WG1 (a1), WG2 (a, b), WG3 (a), WG4 (a1,b) Election of second year COC
Year 1 Month 10-12	Approving second round of STSM applications year 1
Year 2 Month 1-3	First MC meeting and WG meeting in second year
Year 2 Month 4-6	Approving first round of STSM applications year 2
Year 2 Month 7-9	Organization of second MC/WG meeting during annual meeting Achieving WG objectives WG1 (b1), WG2 (c, d), WG3 (b), WG4 (a2,b) Election of second year COC
Year 2 Month 10-12	Approving second round of STSM applications year 2
Year 3 Month 1-3	First MC meeting and WG meeting in third year

Year 3 Month 4-6	Approving first round of STSM applications year 3
Year 3 Month 7-9	Organization of second MC/WG meeting during annual meeting Achieving WG objectives WG1 (a2, c, d), WG2 (e, f), WG3 (c), WG4 (a3,b) Election of fourth year COC
Year 3 Month 10-12	Approving second round of STSM applications year 4
Year 4 Month 1-3	First MC meeting and WG meeting in fourth year
Year 4 Month 4-6	Approving first round of STSM applications year 4
Year 4 Month 7-9	Organization of second MC/WG meeting during annual meeting Achieving WG objectives WG1 (b1, c, e), WG2 (g, h, i), WG3 (d,e), WG4 (a4, c,d)
Year 4 Month 10-12	Approving second round of STSM applications year 4

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT; BE; DE; DK; EE; EL; ES; FR; HR; HU; IE; IL; IT; LT; MK; NL; PL; SE; SK; UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 80 Million € for the total duration of the Action.

This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly."

H. DISSEMINATION PLAN

H.1 Who?

Different and variegated “target audiences” will be identified that may find interest and/or benefit from the dissemination of the results, and deriving recommendations, obtained within this Action network. This is a key aspect of this Action, aiming at translating its research activities into a dissemination strategy, making the best possible use and the most effective exploitation of the results obtained within the network and delivering them to specific targets.

The most obvious one is represented by other researchers actively working in the field or involved in different, although correlated research frameworks. Interactions at this level will allow exchange of information with Research Institutes, Centres of Excellence and Academic Authorities that will result in implemented scientific expertise within Europe.

Reports will be submitted to local, regional and national health Authorities and Policy Makers covering the main points and the major issues emerged from data collected by the different member countries within the network, in order to provide guidelines, implement interactions and promote the development of useful strategies of prevention and management of animal and human health.

Furthermore, a recent survey has clearly shown that EU citizens trust scientists and are very interested in science. However, they do not feel informed enough and would like scientists to tell them more about their work (*European Commission Eurobarometer, published June 2010*). This is why this COST Action will pursue a dissemination strategy that will fill the gap between specialised research activities within the Action and non specialised people, in an attempt to more closely connect science to general public.

H.2 What?

Besides the preparation of proceedings, manuals and conventional scientific publications in peer-reviewed Journals, this Action will aim at translating its research activities into a dissemination strategy, making the best possible use and the most effective exploitation of the results obtained and delivering them to specialised as well as non–expert audiences. This is a key aspect in order to keep people up to date with contemporary developments in research and to give the public opportunities to discuss the potential impact of scientific networks on society, sharing awareness and responsibilities in decisions where science and technology play an important role.

H.3 How?

The Action research activities will be translated into a dissemination strategy that will allow contacts with the press in order to explain the key aspects of the research. Organization of workshops, seminars, and conferences will implement scientific discussion among the MC while the active participation to national and international conferences will be encouraged to increase interactions with other researchers working in the field, circulate information and increment visibility of the Action. Newsletters will be prepared and circulated among members in order to encourage collaboration and synergies within the participating countries/laboratories.

During this COST Action, a dedicated website will be generated to post general information for the public. An open discussion will be implemented through the use of social media by preparing a Facebook account that will allow the set up of an electronic communication network with fast, real time, low cost connection within the Action (with a password) and between the Action and general users (open).

The distribution of informative leaflets and non technical publications will be very important in this COST Action, displaying the Action achievements and making them available to the general public as well as at national and international events.

On special events a "café scientifique" will be set up to approach the public in a relaxed, informal atmosphere and to encourage discussion and feedbacks on selected topics related to the Action.

Other means to disseminate results include press releases, an internet discussion forum on the Website, scientific publications, non-technical publications, the workshops and conferences and related proceedings, the national and international conferences, the Short Term Scientific Missions and the teaching activities of scientists involved in this COST Action.