Department of Animal Reproduction

The Department of Animal Reproduction hosts 7 independent research groups working in closely interwoven research areas, with the goal of understanding the fundamental processes in reproduction and improve reproductive efficiency. Research includes a broad range of species and incorporates whole animal experiments, cell and tissue culture techniques, and molecular biology and genetic studies. The Department takes a multidisciplinary approach to conduct research on different aspects of the reproductive physiology and technology in several animal species. A large number of disciplines work together to comprehend the mechanisms that control fertility and embryo development, to preserve the genetic resources and the biodiversity, to promote the animal welfare, and to optimize the animal production. The research at the Department also focuses on new reproductive biotechnologies and aims at improving the sustainability of livestock farming and at evaluating the impact of environmental changes on reproductive efficiency. The multidisciplinary activities are taken by different research groups; the researchers approach includes physiology, pathology, and technology reproductive in different animal species. The work focuses on developing innovative practices in animal reproduction and subsequent promoting of research transference to satisfy the demands on livestock production.

The Department has two singular facilities: The Experimental Farm, which includes livestock species (swine, sheep, goat, rabbit, and poultry), wild species (mouflon, Iberian ibex) and laboratory animals (mouse, hamster, etc); and a Genetic Resource Bank (germplasm, embryos, and somatic cells) from INIA, which includes genetic samples from endangered native breeds, wild game species, and endangered/extinct wildlife species.

RESEARCH GROUPS

- Physiology and technologies of reproduction in small ruminants
- Gametogenesis, molecular embryology and transgenesis
- Assisted reproduction and preimplantation embryology in bovine
- Physiology and technology of reproduction in swine
- Comparative Physiology
- Animal parasitology and reproduction
- Animal genomic engineering

HEAD OF DEPARTMENT:

Alfonso Gutierrez Adan
secra@inia.es
The aims of the group are reflected in two research lines. In our work with wild species (firstly wild ruminants but also other wild species, including avian species) our aims fall under the headings of their reproductive physiology and its application in conservation (germplasm banks) and sustainability (hunting performance). In our work with domestic small ruminants our aims are focused on the development of alternative methodologies for the control and management of reproduction, avoiding the administration of exogenous hormones. Other aims are the comparative spermatology and cryobiology, the oogenesis in prepupal ewe lambs and their relation to the acquisition of oocyte competence and embryo development.

Development activities transfer and dissemination of synchronization protocols for fixed time artificial insemination in goats; embryo production and freezing in goats and ewes; these activities involve cooperation projects, contracts and courses in Universities and Research Centres. Transrectal ultrasound-guided massage of the accessory sex glands (TUMASG) is an alternative technique, recently developed in our laboratory, to collect sperm, which requires few electrical stimuli or none at all. We have studied several indicators of stress and have concluded that sperm collection by TUMASG is less stressful than electroejaculation without altering sperm characteristics in conscious goat bucks. The development of procedures like freezing at high cooling rates or vitrification might be extremely useful when working with wild ruminants. We successfully described sperm vitrification from many species. Our findings revealed that vitrifying-warming reduced the sperm head size in a similar fashion than freezing-thawing. Iberian ibex sperm vitrification resulted in good quality spermatozoa with an adequate in vitro fertilizing ability. During cryopreservation, sperm cells are exposed to cold shock and atmospheric oxygen. This leads to the overproduction of ROS and increases the susceptibility of the sperm cell membranes to lipid peroxidation. The elimination of debris, dead spermatozoa and other cells by selective washing methods helps to avoid too high an increase in the concentration of ROS and improve the motility variables and viability of cryopreserved sperm. We have compared the classic washing method and density gradient centrifugation (DGC) in goat semen for respects. DGC was associated with overall better values for sperm motility parameters measured after chilling and freezing-thawing. DGC appears to select non-capacitated goat sperm.


Our major research focus is to understand the genetic and epigenetic mechanisms that control the early embryo development in vivo and in vitro; analyse the impact of preimplantation development for fetal development and adult; analyse the mechanisms of in vitro sperm selection; and determine the role of mRNA splicing on regulation of embryo development, sex determination, and fertility.

In the 2016-2017 period, we investigated in the spermatozoa the mechanisms of sperm thermotaxis related with the hyperactivation and how thermotaxis can select in vitro the sperm that is selected in vivo; demonstrating that this sperm selection improve the quality of the embryo and implantation rate. We have also reported that spermatozoon telomeres determine telomere length in early embryos and offspring; and we have found that the elimination of methylation marks at lysines 4 and 9 of histone 3 (H3K4 and H3K9) of spermatozoa alters offspring phenotype.

In relation with oocyte, we have determined the exocannabinoid’s effect on in vitro bovine oocyte maturation via activation of AKT and ERK1/2. In relation with gametogenesis, we have determinate the role of two splicing factors, Zrsr1 and Zrsr2, on spermatogenesis and oogenesis, respectively. These factors are essential for minor splicing, indicating that noncanonical splicing are essential for meiosis of male and female gametes. Also both splicing factors are essential for preimplantation development, critical in early stages for embryo genome activation. We have also demonstrated that oviduct fluids affects the epigenetic landscape of the embryo. The culture of bovine embryos with oviduct fluid induced DNA methylation changes in specific genomic regions in resulting blastocysts, indicating that by including molecules secreted by the oviduct to the media used for the incubation/culture of both gametes and embryo, the adverse periconceptional environment in in vitro derived embryos could be reduced. In relation with transgenesis, we have developed a new system to produce transgenic mice using microinjection of spermatids in the oocyte (ROS1).


Our principal research focus is to analyse the early embryonic development in vivo and in vitro in mammals; determine the factors affecting in vitro embryo production; understand the mechanisms controlling maternal-embryonic interactions and the quality of the embryo; develop different in vitro models for the analysis of embryo maternal communication; study factors responsible for infertility in dairy cows and develop strategies to reduce embryonic losses and increase pregnancies; and study the freezability by vitrification of in vitro bovine embryos and their direct transfer on recipients.

In the 2016-2017 period, we studied embryo-maternal crosstalk in the oviduct of the cow in vivo and in vitro. Regarding the in vivo experiment, we evidenced transcriptomic differences within the oviduct (in the bovine oviductal epithelial cells, BOEC) induced by the presence of an embryo. Under our experimental conditions it seems that we have succeed to describe the effect of a single embryo in the maternal tract, increasing our knowledge related with the communication established between them in physiological conditions. We have also conducted various in vitro experiments to test the results observed in vivo. Moreover we aimed to observe if the in vitro transcriptomic response of BOEC to the early embryo were the result of a contact-dependent signaling effect or interactions with embryo secretions. We found that the stage of early embryo differentially affects the transcriptome of BOEC in vitro, although, these changes may be related either with direct embryo contact or embryo secretions released into the media. Furthermore, we demonstrated that the use of low concentrations of oviduct and uterine fluids in in vitro culture supports embryo development, and improve blastocysts.


The principal objective proposed by our working group has been the study and application of assisted reproduction techniques to reach a greater genetic projection in pig production centers. In this context, the following work lines have been developed: Assessment of the ambiental temperature increase effect on male reproductive function; Addition of cholesterol to the sperm plasma membrane to improve its viability in conservation systems; and the analysis of rearrangement chromosomal imbalances on the fertility and litter size.

In order to assess the effect of the ambient temperature increase on the reproductive capacity of boars, a group of pigs were housed in facilities with a temperature 5ºC higher than the animals of the control group. The study started in the prepubertal phase (3 months) and ended when all the animals reached puberty (7 months). There were no differences between groups in testicular morphometry, serum testosterone concentration and structure of the seminiferous tubules. However, there was a significant improvement in seminal quality in those animals housed with lower temperature that showed better motility, acrosomal structure, and maturity state of the sperm cells.

In order to improve the viability of spermatozoa subjected to cryopreservation. The structure of the spermatozoa plasma membrane was modified, by the increasing of cholesterol content in the membrane bilayer structure. The methodology used was the incubation of spermatozoa before freezing, with cyclic oligasacids (cyclodextrins) that are capable of catching cholesterol molecules. Unlike what happens in other animal species such as stallions, bulls, rams and goats in which colesterol addition has showed positive effects, in pig thawed samples, no positive effects were observed on the sperm viability in those samples in which cholesterol was added.

The constitutive heterochromatin in the chromosomes plays a fundamental role in the genome stability, in cell division processes in the mitosis and in gametes meiosis. The variations that this type of chromatin can experience, are evaluated by the presence of C-band polymorphisms in chromosomes and they have shown an important incidence on reproductive parameters in humans. In our work, on a population study carried out in Iberian pig, the incidence found of this type of alterations was 10.16%, but none of the boars that presented this type of anomaly showed a decrease in the reproductive parameters compared to animals with normal karyotype.
The awareness of factors determining morphological and physiological phenotypes has evolved in the last years from genome to a more complicated concept (developmental programming) in which prenatal and postnatal environmental conditions markedly modify the epigenotype, and therefore the phenotype and homeostasis of the individuals, and determine partly, their juvenile growth, life-time fitness and disease risks. These processes are maintained in different mammal species; this fact allows the selection of the most optimal animal model depending on the research objectives. Hence, the objective of the group is to study the developmental origins of physiological/pathological features of mammalians by using rabbits, ruminants and swine as models.

The scientific activities are currently focused on a) optimization of fertility yields of methods for reproductive management in ruminants; b) adaptation of image techniques to the animal models used (mainly, endoscopic and ultrasonographic techniques); c) characterization of models based on rabbits, sheep and pigs for the study of placental pathology and intrauterine growth restriction (IUGR) under maternal malnutrition; d) assessment of factors (fetal genotype and sex) modulating the effects of uterine environment (in terms of supply of oxygen and specific nutrients to the conceptus) on fetal development and occurrence of IUGR.

**MATERNAL METABOLISM**

- Cholesterol and triglycerides
- Sex-related better transfer of essential fatty acids
- Sex-related greater synthesis of non-essential fatty acids

**SELECTED PUBLICATIONS**


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Our major research focus is to understand the epidemiology of parasitic diseases, the development of new diagnostic techniques as well as evaluation of systems of parasite control.

We also study the influence of parasitic diseases in animal reproduction and the design of control programmes against parasitic diseases (zoonotic or not) and arthropod vectors.

In the 2016-2017 period, we have successfully applied a new integrated model to control three host ticks under field conditions. We also try to know the existence of resistance to the acaricides and characterize them if they are present.

We have implemented alternative methods in animal research as in vitro feeding of hard ticks and tick cell cultures. This novel technique represents a very useful alternative method in animal experimentation that allow us to implement actual screening techniques searching for new products with acaricidal activity or the role of ticks as vectors of several pathogens to human or animals.
Genome modification in animals constitutes a powerful tool to understand the molecular root of diverse physiological and pathological processes, including those involved in animal reproduction. The main aims of our research are:

- Develop CRISPR technology for genome modification in livestock species.
- Understand conceptus elongation in ungulates by gene editing and in vitro approaches, in order to develop nutritional and pharmacological approaches to prevent reproductive losses in farm animals.
- Understand reproductive processes by genome edited models.
- Develop novel methods for selecting the sex of the offspring, applicable to farm animals.
- Understand the epigenetic basis of mammalian phenotype, its inheritance, and the alterations caused by Artificial Reproductive Techniques.

Rabbit embryos with (wt) or without (KO) ZP4 protein. KO embryos show a thinner and irregular zona pellucida (B) and do not develop properly to the expanded blastocyst stage (C).

Sequencing reaction of a PCR product showing several alleles generated by CRISPR.