DESCRIPTION FOR THE GENERAL PUBLIC

In dairy cows the occurrence of twin pregnancies results in financial loss, however, in breeds of beef cattle, the occurrence of twin pregnancies is a desirable feature. Ovulation is the critical process in reproduction leading to the release of oocytes from ovarian follicles. After ovulation, during the early luteal phase, the oocyte is available to be fertilized by sperm. The factors which govern the subtle links between follicle development, oocyte quality and follicle loss remain unclear despite extensive studies undertaken to understand the critical patho-physiological and molecular mechanisms that underlay the accelerated decline in follicle functions and numbers.

Due to the low heritability, the long intergenerational period and the unfavourable correlation with milk yield, occurrence of twin pregnancy is difficult to control by genetic selection in farm animals including cows. Insulin growth factor (IGF-1) has a significant effect on the occurrence of twin pregnancies. It has been shown that IGF-1 plays an essential role in bovine reproduction. IGF-1 influences fertility at different levels within the hypothalamic–pituitary–gonadal axis. In the ovaries, IGF-1 and its receptor (IGF-1R) are selectively expressed in granulosa cells of healthy and growing ovarian follicles. However, there are no systemic and conclusive data describing the mechanisms leading to twin / double ovulation pregnancies. It becomes necessary to know, at the cellular and tissue level, the mechanisms controlling ovulation and atresia of ovarian follicles, the relationship between local regulatory factors, including transcription factors, growth factors and hormones, which at local level can control development, maturation, and ovulation predisposition to double ovulation.

Mathematical modelling of bovine reproduction can be used to verify hypotheses, to predict the responses to different treatments and to reduce the need for future testing. In predicting reproductive indicators/markers, it is necessary to develop mathematical models describing the relevant mechanisms that control the development, recruitment, ovulation and aging and atresia of ovarian follicles, and consequently lead to double ovulation and predisposition to twin pregnancies. Data for mathematical analysis will come from large-scale *in vitro* and *in-vivo* studies carried out using modern omnics analysis (genomic, transcriptomic and proteomic).

A system modelling approach involving mathematical relations to analyse experimental data, provides an integrative pathway to examine the complex interactions between distinct biological entities (genes, hormones, miRNA). Models will be used to describe, reproduce, and predict the course of these interactions in abnormal and normal follicular function not only in cattle, but will also as the basis for similar models in other animal and human species. In biological experiments, the effect of IGF-1 on the growth and function of granular ovarian follicle cells *in vitro* will be determined. In addition, the genes and proteins associated with the necropsy process (a new type of cell death), characteristic of immunological processes, will be tracked. Additionally, in order to find a new marker(s) of the quality of follicles and indicators of double ovulation, the expression of non-coding RNAs, the so-called miRNAs, will be examined and proteomic analysis will be carried out. The results of the hormonal determinations, on transcriptomic as well as proteomic levels can be used to explain the phenomenon of double ovulation and twinning in cows. The results from the *in vitro* experiments will be compared in the *in vivo* situation as a gold standard. The results of all molecular biology experiments will be used to establish and evaluate the planned mathematical model to predict earlier mentioned ovarian events, especially double ovulation.

Analysis of the results from *in vitro* and *in vivo* experiments will lead to the creation of a mathematical description of ovarian follicle cell function, which will include for instance an explanation of IGF-1 effects on transcriptional factors, enzymes and hormones involved in the regulation of follicle development and death. Interactions between regulatory factors at the tissue, cell, receptor, as well as gene level, including transcription factors (miRNAs), secretion and action of agents and their biological effects, will be described mathematically to predict changes in expression of regulatory factors.