A differential equation model to investigate the dynamics of the bovine estrous cycle

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Abstract

To investigate physiological factors affecting fertility of dairy cows, we developed a mechanistic mathematical model of the dynamics of the bovine estrous cycle. The model consists of 12 (delay) differential equations and 54 parameters. It simulates follicle and corpus luteum development and the periodic changes in hormones levels that regulate these processes. The model can be used to determine the level of control exerted by various system components on the functioning of the system. As an example, it was investigated which mechanisms could be candidates for regulation of the number of waves of follicle development per cycle. Important issues in model building and validation of our model were parameter identification, sensitivity analysis, stability, and prediction of model behavior in different scenarios.

Fertility in dairy cows

Bovine fertility is the subject of extensive research in animal sciences, especially because fertility of dairy cows has declined during the last decades. Subfertility has negative implications for dairy farm profitability, sustainability of animal production and animal welfare, as it takes more time and effort to get cows to be pregnant. The decline in fertility has coincided with selection for a higher milk yield, and is manifested in alterations in hormone patterns, reduced expression of estrous behavior, and lower conception rates. However, it is unknown if and how high milk yield and subfertility are causally related. Systems biology approaches, including the use of mathematical models, can help to increase our understanding of the complex interplay of factors involved in the reproductive cycle. Such models can be very valuable in studying effects of e.g. stress or disease on reproduction [1].

The bovine estrous cycle is the hormonally controlled recurrent period when the cow is preparing for reproduction by producing a fertilizable oocyte. The main tissues and organs involved in the regulation of the estrous cycle are the

ovaries, the uterus, the hypothalamus and the anterior pituitary. These organs interact via hormones in the blood. A normal cycle includes two or three wave-like patterns of follicle development, in which a cohort of follicles starts to grow. The length of the estrous cycle is often taken to be approximately 21 days, but the cycle length may be shorter in two-wave cycles than in three-wave cycles. The first one or two waves produce a dominant follicle that does not ovulate, but undergoes regression under influence of P4 (see abbreviation key in the caption of Fig. 1.). The dominant follicle in the last wave produces increasing amounts of E2, triggering the surge of LH, which induces ovulation. Once an oocyte is successfully ovulated, the remains of the follicle form a new P4-producing CL.

In this chapter we briefly describe the development of a mathematical model of the bovine estrous cycle, we discuss how such a model could be validated, and we show an example of how the model can be used to investigate patterns of follicle development. The model summarizes physiological knowledge and empirical data, and thereby provides insight in the regulatory structure of the system.

Modeling the bovine estrous cycle

The endocrine and physiologic regulation of the bovine estrous cycle has been studied extensively. For some specific mechanisms or parts of the system mathematical models have been developed (reviewed in [2]), but mostly these models were of limited scope and do not contain all the major tissues and hormones necessary for simulation of the dynamics of follicle development over consecutive cycles. From the mathematical point of view, many biological processes, such as hormonal interactions, can be modeled with the help of differential equations, which describe the rates of change of the involved substances over time. We developed a mathematical model of the dynamics of the bovine estrous cycle on individual cow level that is able to simulate follicle and CL development and the periodic changes in hormones levels that control these processes by a set of linked differential equations. We performed an extensive literature research on how the individual components of the cycle function together, obtained abstraction levels that display the most important mechanisms, and constructed a flow chart of their interactions. The key components of the biological system and their interactions incorporated in the model are shown in Fig. 1.

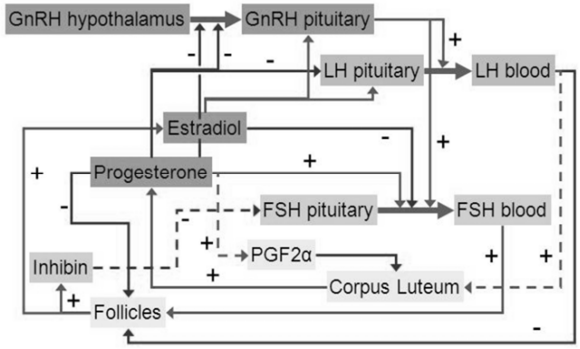


Fig 1. Key components of the biological system and their interactions. '+' and '-': inhibiting and stimulating effects respectively. Dashed lines: time delay.

Abbreviation key:
Foll: follicular function (in the model representing the combined capacity of all follicles present at any time to produce E2 and Inh)
CL: corpus luteum (in the model representing the capacity of the CL to produce P4, rather than the physical size of the CL)
P4: progesterone
E2: estradiol
Inh: inhibin
GnRH: gonadotropin releasing hormone
FSH: follicle stimulating hormone
LH: luteinizing hormone
PGF2α: prostaglandin F2α

We derived a differential equation for each of the components (boxes) mentioned in Fig. 1. This initial model contains 12 ordinary and delay differential equations and 54 parameters [3] and is partly based on previous work by Selgrade and colleagues [4] and Reinecke [5] on modeling the human menstrual cycle. Hill functions are used to model the non-linear stimulating and inhibiting effects of hormones. In the model, the amount of GnRH in the hypothalamus is a result of synthesis in the hypothalamus and release into the pituitary and is affected by P4 and E2. FSH is synthesized in the pituitary when the level of Inh is low. FSH release is stimulated by GnRH and inhibited by E2. LH synthesis in the pituitary is stimulated by E2 and inhibited by P4, and LH release is stimulated by GnRH. Follicle development is stimulated by FSH and inhibited by P4 and the LH surge. The production of P4 is proportional to CL function. PGF2α induces CL regression and is stimulated by P4 with a time delay. The production of E2 and Inh is proportional to follicular function. Simulation results (Fig. 2) show that a set of equations and parameters was obtained that describes the system consistent with empirical knowledge. Even though the majority of the mechanisms included

in the model are based on relations that in literature have only been described qualitatively, the model output is surprisingly well in line with empirical data.

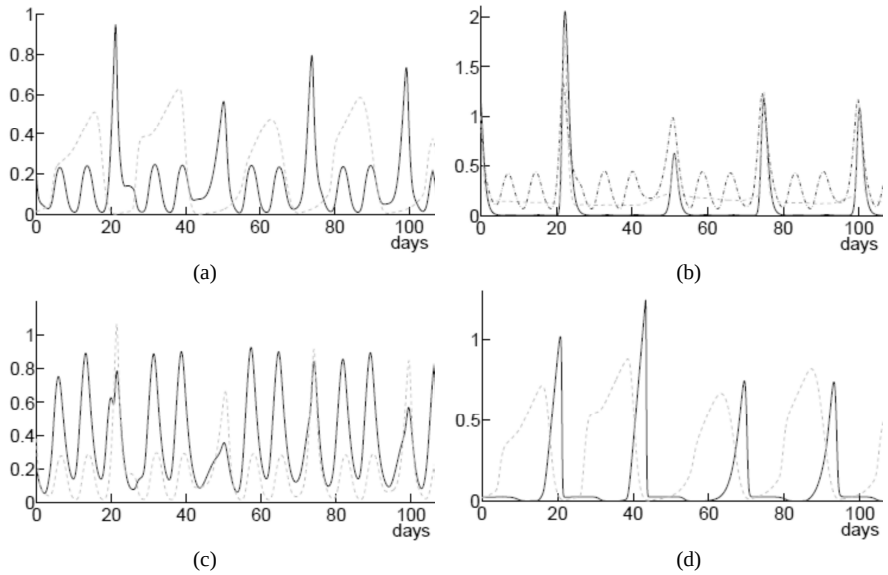


Fig 2. Model parameterization generating estrous cycles of approximately 21 days, with three peaks of FSH and three corresponding waves of follicular growth. The third wave of follicular growth takes place when P4 levels are low, which results in increasing levels of E2. This causes an LH surge, which then triggers ovulation. (a) Foll (solid line) and CL (dashed line). (b) GnRH (solid line), LH (dashed line) and E2 (dashed-dotted line). (c) FSH (solid line) and Inh (dashed line). (d) PGF2α (solid line) and P4 (dashed line). The equations are expressed on a relative scale in order to simplify parameter estimation, and therefore the y-axis of the figures is dimensionless.

Model validation

There is no general procedure for model validation. The most important aspect is whether certain model simulation outcomes match with some given experimental data. Model validation therein aims to assess the predictive accuracy of the numerical model, and thereby to build confidence in the model. A model has an added value when it not only matches given data, but also gives insight into certain processes that cannot be observed by measurements, and thus hints to explanations for certain phenomena. Here we discuss four steps that we consider to be important for the model building and validation of our specific model of the bovine estrous cycle: parameter identification, sensitivity analysis, stability, and prediction of model behavior in different scenarios.

Our model describes the interactions between key components of the bovine estrous cycle. For solving the system of differential equations, the solver RADAR5 [6] developed for the solution of stiff delay differential equations was

used. The main difficulty lies in the identification of the involved parameters. Most parameter values in the model are neither measurable nor available in literature, and sometimes even the range of values is completely unknown. For a model of a complex system with various components functioning together, this leads to a large number of differential equations and unknown parameters. Under these circumstances, estimating all parameters simultaneously is impossible. For our model we used a model decomposition approach to obtain a good initial guess of the parameter values for the optimization procedure. The model was decomposed into disjoint model parts, and parts of the model were temporarily replaced by input curves based on published data of hormone profiles of cows with a normal estrous cycle. A first subset of parameters was then estimated, and step by step the output functions for the other model parts were fitted, until finally a closed network was obtained [7]. Parameters were estimated with software developed at the Zuse Institute (NLSCON). This software uses subtle mathematical techniques such as affine covariant Gauss-Newton methods that take into account sensitivities and linear dependencies of the parameters [8].

A sensitivity analysis for the complete set of model parameters has been performed with techniques described in [8]. A higher sensitivity means that a change in the value of the parameter has a larger effect on the model solution. Sensitivity analysis can therefore identify the parts that need a more precise parameter estimation. It is an important step not only in the parameter estimation algorithm, but also in model validation, since it quantifies the relative importance of parameters. Thereby it shows if the model does not depend unexpectedly strong on biologically less relevant parameters. The sensitivity analysis of our model confirmed that parameters that are very important for follicle development and cycle length had a high impact on the model solution.

Model validation also deals with the question of stability. Stability investigates how changes in the model input affect model output. In a stable model, small perturbations should not disturb the qualitative behavior of the system. As can also be observed in Fig. 2 and 3, some parameterizations of our model produce a stable limit cycle (periodic behavior), while others generate consecutive estrous cycles that are not entirely identical (quasi-periodic behavior). The variations between simulated cycles are thus not an intrinsic characteristic of the model, but depend on the parameterization. In the bovine, a new population of follicles is recruited in each cycle, with a different number and size, leading to differences in the hormonal profiles that are the result. We therefore think the variation between estrous cycles is not only due to changes in external factors for that cow, but also arises from the fact that each cycle presents slightly new and somewhat different ‘starting values’ for the next cycle, which we think that our model can mimic. Stability of the model is also an essential requirement to handle

variation between individuals. With one single model we aim at finding parameterizations for individual measurement data. This could be done by defining input functions of individual time series, but also by simulating external influences like effects of nutrition or stress. However, experimental data available in literature often do not meet the requirements for these individual parameterizations, because either the time scale of investigation is too short, or the data lack information of certain experimental parameters.

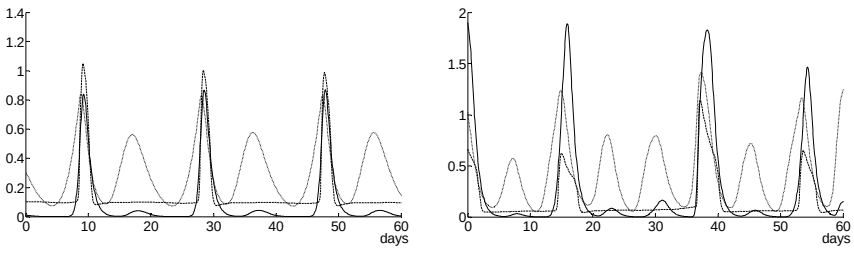
Apart from fitting to individual data, the model could be used to determine the level of control exerted by various system components on the functioning of the system. This could be done by changing the value of specific parameters, aiming to obtain a certain model output, or by mimicking e.g. external hormone administration. Experimental data to verify the predicted causes of certain phenomena are not always available, but the simulation could provide some likely candidates involved in the regulation of certain mechanisms that could be tested in further experiments. Further, the model can serve as a basis for more elaborate models and simulations, with the ability to study effects of external manipulations and genetic differences. Summarizing, there are many possible model applications, and therefore we should think carefully about what we want to investigate and which parts of the model need therefore to be validated.

Using the model to investigate patterns of follicle development

The model was initially parameterized to generate three waves of follicle development per cycle. One model application that has already been performed was to investigate which mechanisms could be likely candidates for regulation of the number of waves in the bovine estrous cycle. This specific research question allowed to predict the temporal behavior of the system, to optimize parameters, and to study the sensitivity of dynamical processes with respect to its initial parameter values. A normal bovine estrous cycle contains two or three waves in which a cohort of follicles start to grow. However, the reason for cycles being of the two or three waves type is unclear. Some studies report better fertility in three-wave cycles compared to two-wave cycles [9], and it has been suggested that the older and larger ovulatory follicles in cycles with two waves contain oocytes of less quality than cycles with three waves [10]. However, other studies showed no difference [11]. A better understanding of endocrine mechanisms regulating follicle development is important to obtain more precise control of the estrous cycle, which can help to improve pregnancy rates. In the bovine, the follicle that is dominant at the moment of CL regression develops to become the ovulatory follicle. We assumed that there may be two mechanisms by which the follicle wave pattern can be influenced. One is the rate of follicle growth and the other is the time point of CL regression. In our model, follicle growth is stimulated by

FSH and inhibited by P4. Therefore, the first mechanism might be induced by changing the effect of FSH or P4 on follicle growth, or by changing FSH or P4 synthesis. The second mechanism, i.e. the time point of CL regression, is expected to have an effect on the follicular wave pattern because two-wave cycles can occur when the CL starts to regress at an earlier time point, e.g. because of an earlier increase of PGF2α. We have selected ten parameters in our model that relate to these two overall mechanisms, and we have tested whether changing the value of these parameters affects the number of waves per cycle in the model simulations. For this purpose, the model was extended with an extra equation, which is described in detail in [12]. In brief, the fixed time delays for the effect of the increase in P4 levels on PGF2α release (which limited the predictive ability for this part of the model) were replaced by a mechanism in which the ability to synthesize PGF2α develops over time under influence of P4. PGF2α levels now rise because P4 stimulates the production of enzymes and receptors required for PGF2α production, which was previously included as a 'black box' by using large delays.

Simulation results showed that a change in the value of specific parameters involved in the regulation of follicle growth rate or the time point of CL regression can change the number of waves in a cycle (Fig. 3). Of the ten parameters tested, six affected the number of waves per cycle. Like in real cows, the period of oscillations (cycle length) appeared to be variable. Cycles with two waves had a shorter cycle length. In non-ovulatory waves of two-wave cycles, FSH levels were higher, Foll (follicular capacity to produce E2 and Inh) was larger, and therefore also E2 and Inh levels were higher compared to non-ovulatory waves of three-wave cycles. The two-wave cycles obtained by a change in follicle growth rate were due to a later emergence of the second wave, while the two-wave cycles obtained by a change in time point of CL regression were caused by a shorter CL life span.



(a) (b)

Fig 3. A change in specific parameter values can result in a series of 2-wave cycles (a) or alternating 3- and 2-wave cycles (b). E2 (dotted line), GnRH (solid line) and LH (dashed line). This figure was obtained by decreasing the parameter that represents the maximum inhibiting effect of P4 on follicular function.

The simulation results thus showed that several components of our model of the bovine estrous cycle can affect the pattern of follicle growth, and some of them are plausible biological mechanisms that could explain these patterns. The model appeared to be sufficiently stable when simulation of two-wave cycles was performed. A reason of poor reproductive performance could be suboptimal matching of follicle growth rate and the time point of CL regression. An earlier time point of CL regression (and therefore a shorter cycle) induces a switch from three to two waves, because when P4 levels are sufficiently decreased at the second wave, this will become the ovulatory wave. Although in the bovine two-wave cycles are on average shorter than three-wave cycles, the difference is not the duration of a complete wave. Based on reported differences in follicle development, we think that differences in number of waves in natural estrous cycles may rather be due to changes in the mechanisms regulating follicle growth rate, and that the shorter cycle length is rather the result than the cause of the change in wave pattern.

In conclusion, this mathematical model can provide plausible pathways of interactions of follicular and endocrine dynamics that contribute to bovine fertility. Our aim is not to develop a model as simple as possible, but a model that, although with a high level of abstraction, includes all the main processes that are considered important from a physiologic point of view, in order to obtain a model that improves insight in these processes.

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