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Advances in modelling of the bovine estrous cycle: Administration of PGF2α

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Abstract

Our model of the bovine estrous cycle is a set of ordinary differential equations which generates hormone profiles of successive estrous cycles with several follicular waves per cycle. It describes the growth and decay of the follicles and the corpus luteum, as well as the change of the key substances over time. In this work we describe recent improvements of this model, including the introduction of new components, and elimination of time delays. We validate our model by showing that the simulations agree with observations from synchronization studies and with measured progesterone data after a single dose administration of synthetic prostaglandin F2α.

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Introduction

The model of the bovine estrous cycle as introduced in [1] contained 12 differential equations and 54 unknown parameters, generating successive estrous cycles of 21 days with three follicular waves per cycle. In [2] an advanced model with 13 equations and 57 parameters has been used to analyse the 2- and 3-follicular-wave behaviour in bovine. However, during simulations of the administration of hormones, we found that this model still had some shortcomings regarding the rise and fall of the corpus luteum (CL). A replacement of mechanisms involved in ovulation and the refinement of luteolysis became necessary. In this work we overcome the shortcomings and present an improved model with 15 ordinary differential equations and 60 parameters.

To build confidence in the model and to benefit from future extensions of the model concerning concrete medical questions, the starting point of our work was the validation of the current model. Experimental data required for model validation would for example consist of measured hormonal concentrations of healthy, untreated, individual cows at different stages of estrous cycle. Unfortunately, measurements published in literature are rare and do often not meet the requirements for validation; observed time scales are often too small or too coarse, or too few substances are measured. So we set out for alternatives to validate the model and simulated the administration of prostaglandin F2α (PGF2α). In veterinary medicine, PGF2α and its analogues are administered to cows mainly to make use of their luteolytic action e.g. in estrus synchronization protocols. It is known that the sudden rise of this substance at certain stages of the estrous cycle results in an immediate decay of the responsive CL, and an immediate fall of progesterone levels in plasma.

1 Approaches - Estrous synchronization protocols

Protocols of estrous synchronization consist of consecutive administration of different hormones or their analogues following a certain order. They have the goal to synchronize the estrous of individual females in order to facilitate timing of following artificial insemination, indepently of estrous cycle stage at the start of the protocol. They are commonly used in cattle and in other domestic and non domestic species. In [3] the effect of synchronization protocols on follicular development and estradiol and progesterone concentrations in dairy heifers was evaluated. In those protocols cows were treated with different combinations of Gonadotropin Releasing Hormone (GnRH), PGF2α and Progesterone (P4). Our first approach to validate the model of the bovine estrous cycle was to include the synchronization protocols performed in [3] together with therein measured progesterone concentrations of individual cows taken during and after conducting the protocols. In a first step, applications of PGF2α were picked out, modeled and inserted in the cow model at different moments of estrous cycle.

PGF2α is responsable for the onset of luteolysis in the cow. With luteolysis the luteal phase of the cycle ends and a new estrous can take place. PGF2α induces functional luteolysis by reducing progesterone production followed by structural luteolysis with tissue degeneration and cell death [4, 5]. PGF2α is synthetized in the endometrium and released in pulses, regulated by estradiol

(E2), P4 and oxytocin (OT) during the estrous cycle [6, 7, 8]. In veterinary medicine, administration of synthetical analogues of PGF2α (e.g. Cloprostenol, Luprostiol, Tiaprost) or original PGF2α (e.g. Dinoprost) is used for various purposes in the cow such as induction of estrous or synchronization protocols. The effect of the treatment depends on the stage of estrous cycle which determines the responsiveness of the CL on the luteolytic effect of PGF2α [5]. At midluteal stage of the estrous cycle administration of PGF2α leads to luteolysis within a few hours. This results in a decrease of P4 concentration, increase of E2, a peak of the Luteinizing Hormone (LH) and ovulation [9].

Virtual administration of PGF2α to the cow model was conducted on variuos days of the estrous cycle. In a first approach the outcome of the model after PGF2α application was not as expected, which gave us a starting point to improve the model.

2 Advancements in the model

To improve the model with respect to the expected effects of a single PGF2α injection, we introduce some new features which are described in this section. A list of the used Hill functions - sigmoidal functions to model inhibitory or stimulatory effects as described in [1] - can be found in Appendix B. Here H^+ and H^- denote scaled positive respectively negative Hill functions. Parameter values are specified in Appendix C.

In the former model, the equation for the CL described the change of the capacity of the CL to produce P4. For reasons described in Section 2.4, we now interpretate this equation as the development of the size (e.g. diameter) of the CL over the cycle. This is also advantageous as soon as we deal with ultrasound measurements for the corpus luteum. Likewise, the equation for the follicles (Foll) now describes the development of the total size of all follicles.

2.1 Administration of PGF2α

It is known that PGF2α and its analogues have a very short half-life [10, 11]. We therefore need an additional component in the model that falls rapidly. Analogues of PGF2α, denoted $\mathrm{PGF}_{\mathrm{syn}}$ in the following, have an up to three times higher biological activity than original PGF2α [11]. Even low doses of $\mathrm{PGF}_{\mathrm{syn}}$ caused a peak in PGF2α that exceeded the natural level [12]. Due to this high potency of $\mathrm{PGF}_{\mathrm{syn}}$, parameters were identified that lead to a three times higher relative level of $\mathrm{PGF}_{\mathrm{syn}}$ compared to normal PGF2α levels. We model the effect of the synthetical analogue by summing the level of $\mathrm{PGF}_{\mathrm{syn}}$ to the normal PGF2α level.

To model the rise of $\mathrm{PGF}_{\mathrm{syn}}$ in the system, we take a function which is zero before dosing time (t_D), and has a sharp left-skewed peak with maximum shortly after t_D. This leads to a slight delay in the effect of the injection. As suggested in [13], based on techniques described in [14], we take the probability density function of the Gamma-distribution with fixed shape parameter $\alpha = 2$, and inverse scale parameter β leading to a left-skewed curve which has its maximum at $t = \frac{1}{\beta}$. The change of concentration of synthetic PGF2α is calculated

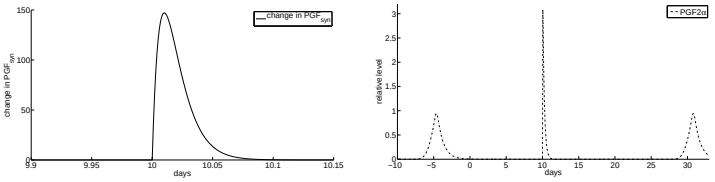
as

$$\frac{d}{dt}PGF_{syn}(t) = D \cdot \beta^2 \cdot t_{mod}(t) \cdot \exp(-\beta \cdot t_{mod}(t)) - c_{PGF_{syn}} \cdot PGF_{syn}(t).$$

The parameter D represents the amount of administered drug scaled to obtain the designated height of the relative level of $\mathrm{PGF_{syn}}$, see Figure 1. The parameter $c_{PGF_{syn}}$ denotes the clearance rate constant of $\mathrm{PGF_{syn}}$. The modified time function t_{mod} is given as

$$t_{mod}(t) = \max(0, t - t_D).$$

The rise of $\mathrm{PGF_{syn}}$ is large right after dosing time and approaches zero quickly thereafter, leading to a rapid decay of the function $PGF_{syn}(t)$.



(a) Rise of $\mathrm{PGF_{syn}}$

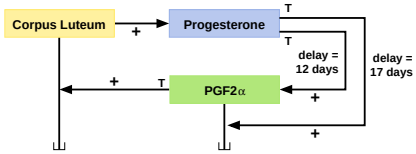
(b) Total PGF2α=PGF+$\mathrm{PGF_{syn}}$ in the system

Figure 1: Administration of $\mathrm{PGF_{syn}}$ at time $t_D = 10$. Parameters are $D = 4$ and $\beta = 100$. Maximum rise of $\mathrm{PGF_{syn}}$ is at $t = t_D + \frac{1}{\beta} = 10.01$. The level of PGF2α is the result of summing $\mathrm{PGF_{syn}}$ levels to naturally arising PGF2α.

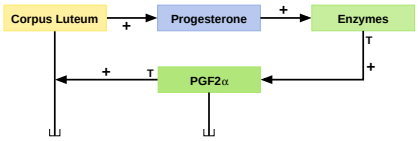
2.2 Luteolysis

In [1], the rise of PGF2α triggering the decay of the CL was modeled as a black box, depending with large delays on P4 only. In [2], this was improved as enzymes were introduced that stimulate PGF2α, and the model became more robust. However, simulating the administration of PGF2α we detected that the modeling of luteolysis still had some deficits. It is known that after the administration of PGF2α the responsive CL decays immediately [15]. In the original model, the CL did not decay fast enough after administration of $\mathrm{PGF_{syn}}$, and neither after rise of the regular PGFα. But since P4 levels, which fall with the CL, should stay on a high level for the duration of the first two follicular waves, we needed the CL to decay later. That means we needed the initator of luteolysis, PGF2α, to appear a couple of days later compared to the original model. To account for this effect, we now model the mechanisms that lead to a rise in PGF2α more precisely. The development of the model regarding luteolysis is illustrated in Figure 2.

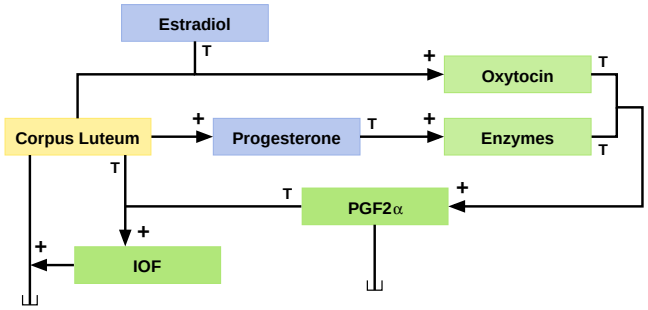
Instead of leaving only the enzymes (Enz) being responsible for PGF2α levels as in [2], we now also include OT as another initiator of PGF2α [16]. E2 stimulates OT synthesis in the granulosa cells [17] and within this the effect of OT on PGF2α [7]. We assume that OT production quadratically depends on CL size, and that it is cleared with constant rate c_{OT}. The equation for the rise



(a) Former mechanisms



(b) Temporary mechanisms



(c) Advanced mechanisms

Figure 2: Changes in the mechanisms involved in luteolysis. '+' marks a stimulatory effect, 'T' denotes that there is Hill function with a threshold involved. No description means a transitition, and '⌞⌟' marks a degraded substance.

and fall of OT is now

$$\frac{d}{dt}OT(t) = H_{17}^{+}(E2) \cdot CL(t)^2 - c_{OT} \cdot OT(t).$$

OT together with Enz are now responsible for the rise of PGFα. With the function $H_{16}^{+}(Enz\&OT)$ which represents a stimulatory effect if the levels of Enz and OT are both high, and the constant clearance rate c_{PGF}, the equation for PGF2α becomes

$$\frac{d}{dt}PGF(t) = H_{19}^{+}(Enz\&OT) - c_{PGF} \cdot PGF(t).$$

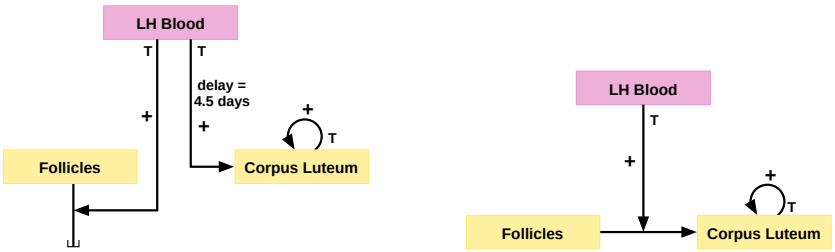
In the former model, PGF2α triggered luteolysis directly, independent of estrous stage. However, it is known that the CL is resistant to the action of PGF2α at early luteal stage. We therefore remodeled the action of PGF2α on the CL. According to [5], the direct action of PGF2α on the CL is mediated by local factors: endothelin-1-system, cytokines, and nitric oxide. The expression of these inter-ovarian substances is upregulated by PGF2α, and strictly depends on the stage of the CL. We introduce a new component to our model and call it inter-ovarian factors (IOF). IOF is stimulated by PGF2α only if the CL has reached a certain size, and cleared with constant rate c_{IOF},

$$\frac{d}{dt}IOF(t) = H_{18}^{+}(PGF\&CL) - c_{IOF} \cdot IOF(t).$$

The rise of the inter-ovarian factors now induces luteolysis.

2.3 Ovulation

In the original model, LH was the initiator of ovulation, responsible for decay of the dominant follicle, and at the same time the initiator of the rise of the CL, 4.5 days after the LH peak. A delay differential equation was needed to model this effect. The atretic follicles were gone from the system, and the CL emerged independently of the size of the just ovulated dominant follicle.



(a) Former mechanisms (b) Advanced mechanisms

Figure 3: Changes in the mechanisms involved in ovulation. '+' marks a stimulatory effect, 'T' indicates that there is a Hill function with a threshold involved. Arrows without description means a transitition, and '⌞⌟' marks a degraded substance.

However, it is known that thecal and granulosa cells of the ruptured follicle transform to small and large luteal cells which form the rising CL [18]. Therefore, to make the model more realistic and to be able to account for different sizes of the dominant follicle [19] we changed the involved mechanisms. The ovulatory follicle now directly influences the initiation of CL growth, and no further delay differential equation is needed. The old and new mechanisms are illustrated in Figure 3. The equations for the follicular size (denoted Foll) and the CL are modified as follows:

$$\frac{d}{dt}Foll(t) = H_{11}^{+}(FSH_{Bld}) - (H_{12}^{+}(P4) + H_{13}^{+}(LH_{Bld})) \cdot Foll(t),$$
$$\frac{d}{dt}CL(t) = SF \cdot H_{13}^{+}(LH_{Bld}) \cdot Foll(t) + H_{14}^{+}(CL) - H_{15}^{+}(IOF) \cdot CL(t).$$

In the model, the part of the follicles decaying due to LH, i.e. the ovulated follicle, is now preserved in the system, forming the rising CL. The scaling factor SF is included to keep the relative levels of the substances between 0 and 1. Further growth of the CL is still modeled by a self-growth, i.e. a positive influence of the CL on its own size from a certain size on. Since the CL therefore starts to grow earlier now, the threshold and rate of self-growth have been adjusted.

2.4 Further modifications

Since the development of the CL depends on three mechanisms (an intiating impulse from LH, a self-growth and the decay caused the inter-ovarian factors), the level of the CL changes as follows: Right after ovulation the CL starts to grow, reaches the size needed for self-growth, and then grows with constant rate until the rise of PGF2α. In the original model, P4 production was proportional

to CL size, and therefore the two profiles looked similar. It is known that P4 production of the CL is not absolutely proportional to the CL size [20]. Data from a study where a single dose of PGF2α was administered showed that the P4 profile stayed low for about 10 days after the PGF2α administration. Therefore, the mechanisms leading to the rise of P4 were adjusted to obtain a P4 production which is lower at start of CL growth compared to later luteal stages. We assume that P4 is quadratically dependant on CL, and change the interpretation of the equation for CL. As mentioned above, the equation for the CL now describes the development of the size of the CL over the cycle, and the production of several substances depends quadratically on CL. The equation for P4 becomes

$$\frac{d}{dt}P4(t) = c_{CL}^{P4} \cdot CL(t)^2 - c_{P4} \cdot P4(t).$$

In the former model, the capacitiy of the follicles to produce E2 and inhibin (Inh) was described in one equation, and E2 and Inh levels were proportional to the relative level of this component. For consistency reasons we now also assume a quadratic relationship between the follicles and E2 respectively Inh, and the corresponding equations become

$$\frac{d}{dt}E2(t) = c_{Foll}^{E2} \cdot Foll(t)^2 - c_{E2} \cdot E2(t),$$
$$\frac{d}{dt}Inh(t) = c_{Foll}^{Inh} \cdot Foll(t)^2 - c_{Inh} \cdot Inh(t).$$

Diminishing the former delay for inhibin on FSH has been possible by augmenting the threshold for inhibin until its negative influence on FSH synthesis arises, at the same time steepening the regulatory effect on FSH. Moreover, the production rate of inhibin as well as its clearance rate have been lowered in order to defer the simulated inhibin curve. The FSH threshold for its influence on the follicles has also been increased. The fact that we were able to dispose this delay without changing the differential equation at all was only possible because the delay was quite short (1.41 days in [2]).

A flowchart of the complete mechanisms of the model is shown in Figure 4.

3 Simulation results

In the advanced model, we do not have delays anymore. Therefore there is no longer a need for a delay differential equation solver. We now use a linear implicit Euler method with extrapolation, implemented in the code LIMEX [21]. Parameters are identified with the software NLSCON developed at the Zuse Institute. This software uses subtle mathematical techniques such as affine covariant Gauss-Newton methods that take into account sensitivities and linear dependencies of the parameters [22, 23].

Our model of the bovine estrous cycle is dimensionless in the sense of [24], i.e. the numerical values of the components are independent of the standard of measurement. Simulated hormone levels and ovarian components have been scaled to be between 0 and 1 by deviding the equation by its maximum output level. Once we have measurement data available we will scale the functions to the corresponding quantities by scaling the involved parameters. This can be done because until now none of the parameters has a fixed value verified

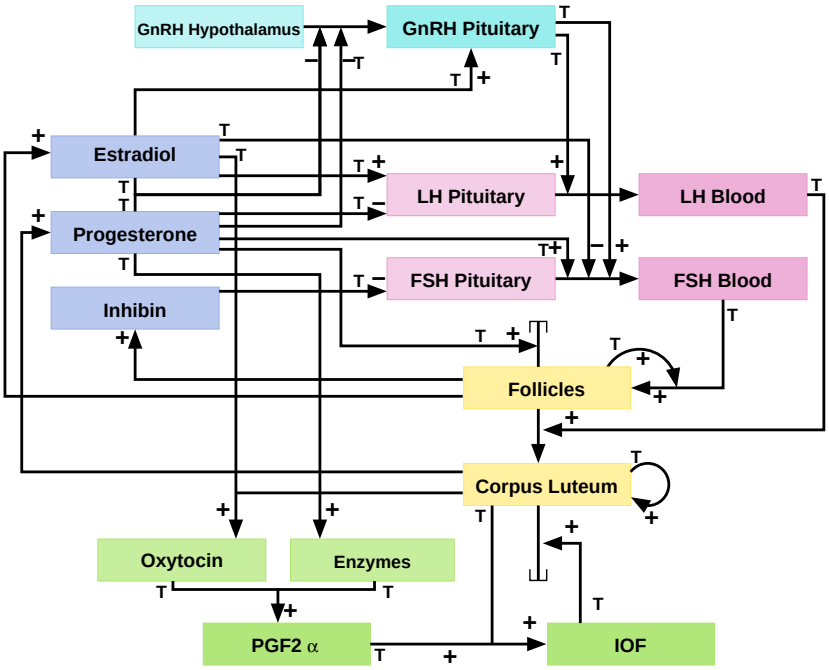
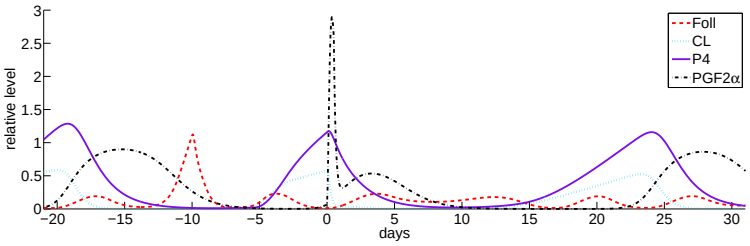
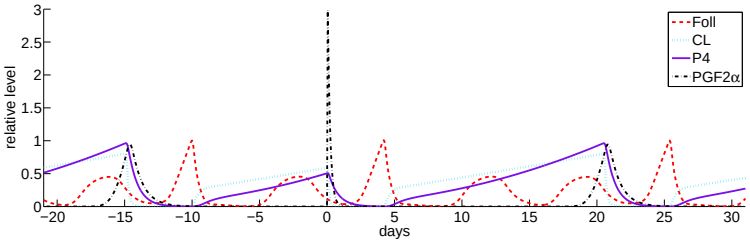


Figure 4: Schematic representation of the compartments in the model of the bovine estrous cycle. '+' marks a stimulatory effect, 'T' denotes that there is Hill function with a threshold involved. No description means a transitition, and '⌴' marks a degraded substance.

by experiments. We refer to the simulated dimensionless output functions as relative level.



(a) Former simulation results



(b) Updated simulation results

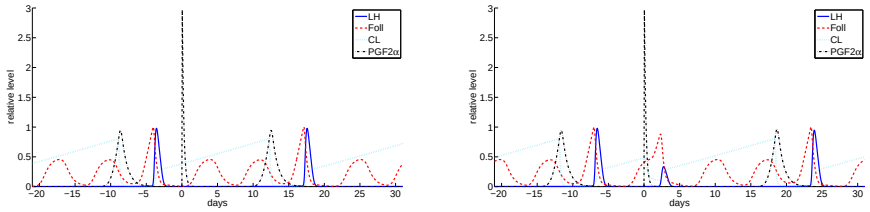
Figure 5: Outcome of the previous model in comparison with current simulations for the substances under particular investigation (Follicles, CL, P4, PGF2α). Day 0 denotes the day of PGF2α admistration. Ovulation has occured 10 days before administration.

The changes in the model described in the previous section have led to the following changes in the simulation results that are shown in Figure 5. In contrast to the previous model, after administration of PGF2α on day 10 after ovulation the CL now decays immediately to zero. P4 levels follow shortly after. Right after administration, PGF2α does not have high levels right anymore, but stays low for 21 days. The most important difference between the outcome of the former and the advanced model can be observed in the follicles. Before, the administration of PGF2α did not affect regular function, anovulatory waves stayed anovulatory. Now, the next arising follicular wave does not decay but continues to rise, leading to ovulation.

In Figure 6 we can observe that virtual administration of PGF2α in the early luteal stage does not lead to a decay of the CL, while at later time points of the cycle it results in an immediate decay of the responsive CL, an LH peak, and ovulation during the following follicular wave.

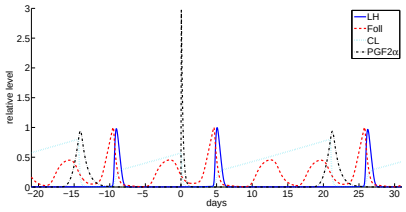
3.1 Progesterone measurements versus simulation after single administration of PGF2α

In a recent study performed at the institute of animal reproduction, faculty of veterinary medicine of Freie Universität Berlin, a single dose of 5 mg PGF2α has been injected to seven cows, and plasma progesterone concentrations have been measured before and after the administration. In particular, blood has

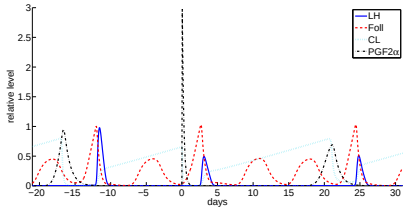


(a) Early luteal phase

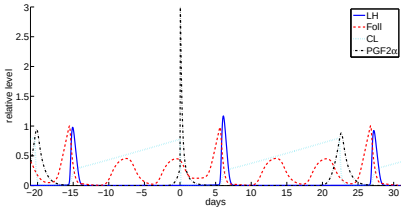
(b) During first follicular wave



(c) After first follicular wave



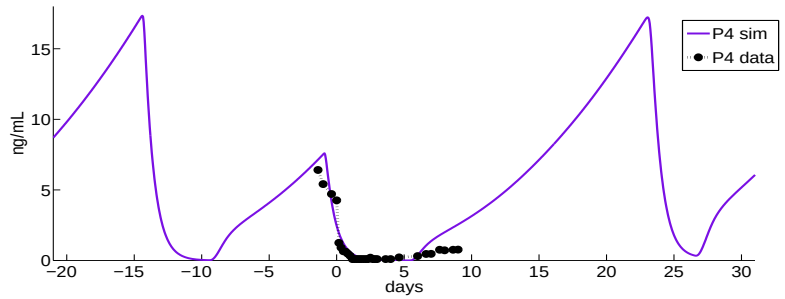
(d) At rise of second follicular wave



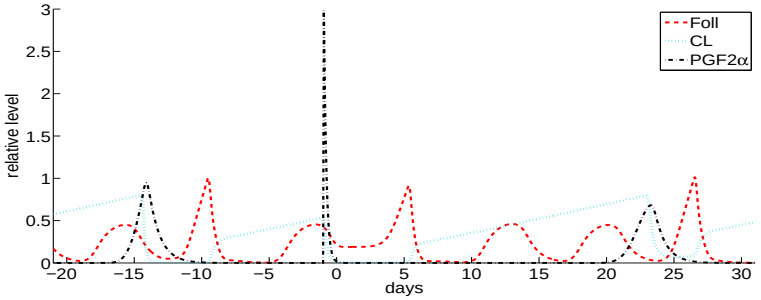
(e) After second follicular wave

Figure 6: Simulation results for the follicles, CL, and LH for administration of PGF2α on different days in the cycle. Day 0 denotes the day of administration.

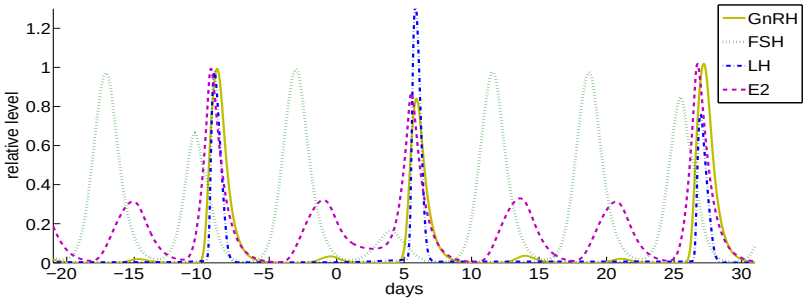
been collected every morning (8:00h) and evening (17:00h) before the injection, every four hours after the injection, and twice a day after ovulation (detected by ultrasound).



(a) Progesterone



(b) PG and Ovaries



(c) Other Hormones

Figure 7: Simulation of a single dose of PGFα. Parameters have been fitted to experimental P4 data.

Parameters have been identified so that the simulated P4 level matches the given data. Note that we now observe and simulate concentrations instead of relative levels for progesterone. Certain parameter units therefore have to be adapted adequately. In Figure 7(a) an example of measured P4 concentrations for one of the examined cows is shown, together with the simulated P4 concentration. Ovulation has been detected by ultrasound a couple of days after the PG injection. This is well captured in the simulation. Not only does this ap-

prove our model, we can also observe substances that are not measured within the experiment, and our simulation gives us insight about the development of these substrates after a single PGF2α injection. For example, in Figure 7(c) we observe a GnRH peak after administration of PGF2α, which can be understood as an increase in pulse frequency and is in the scope of expected observations.

4 Conclusion and Outlook

In this work we have enhanced the model of the bovine estrous cycle which was introduced in [1] and improved in [2]. We have replaced the mechanisms regarding ovulation and refined the modeling of luteolysis. The new components for oxytocin and inter-ovarian factors have been introduced and connected to the rest of the model. To eliminate time delays, certain growth and decay rates, as well as several thresholds and steepness factors have been adjusted. To account for effects observed in experimental data, the relationship between CL growth and the rise of P4 levels has been modified, the action of Foll has been adjusted accordingly.

We have shown simulation results for cows with three follicular waves per cycle. Different parameterizations also lead to cycles with two, four or alternating numbers of follicular waves. The analysis of the underlying dynamics would go beyond the scope of this work.

In the future, the model of the bovine estrous cycle could be used to simulate external influences interacting with the cycle. Possible influences could be stress, negative energy balance, or milk production. Other applications could be the modeling of pathological situations, e.g. cystic ovarian disease, anoestrous, or inflammation. The model could be used to perform a deeper investigation of their interaction with the fertility hormones of the cow. Also, an optimal control problem could be formulated to design synchronization protocols regarding optimal dosing and frequency. A future refinement could require the inclusion of reactions that take place on single-cell level, e.g. receptor binding mechanisms as in [25]. The mathematical know-how for this is under current investigation. The level of detail of the model can be adjusted according to the application. The prospectives and future development will depend crucially on the available experimental data.

Acknowledgements

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Appendix

The model describing the bovine estrous cycle without external manipulation consists of 15 ordinary differential equations with 60 parameters. For virtual administration of PGF2α we use one additional ODE containing three extra parameters.

A Equations

GnRH:

$$\frac{d}{dt} GnRH_{Hypo}(t) = Syn_{GnRH}(t) - Rel_{GnRH}(t) \tag{1}$$

$$Syn_{GnRH}(t) = c_{GnRH,1} \cdot \left(1 - \frac{GnRH_{Hypo}(t)}{GnRH_{Hypo}^{\max}}\right)$$

$$Rel_{GnRH}(t) = (H_1^-(P4\&E2) + H_2^-(P4)) \cdot GnRH_{Hypo}(t)$$

$$\frac{d}{dt} GnRH_{Pit}(t) = Rel_{GnRH}(t) \cdot H_3^+(E2) - c_{GnRH,2} \cdot GnRH_{Pit}(t) \tag{2}$$

FSH:

$$\frac{d}{dt} FSH_{Pit}(t) = Syn_{FSH}(t) - Rel_{FSH}(t) \tag{3}$$

$$Syn_{FSH}(t) = H_4^-(Inh)$$

$$Rel_{FSH}(t) = (b_{FSH} + H_5^+(P4) + H_6^-(E2) + H_7^+(GnRH_{Pit})) \cdot FSH_{Pit}(t)$$

$$\frac{d}{dt} FSH_{Bld}(t) = Rel_{FSH}(t) - c_{FSH} \cdot FSH_{Bld}(t) \tag{4}$$

LH:

$$\frac{d}{dt} LH_{Pit}(t) = Syn_{LH}(t) - Rel_{LH}(t) \tag{5}$$

$$Syn_{LH}(t) = H_8^+(E2) + H_9^-(P4)$$

$$Rel_{LH}(t) = (b_{LH} + H_{10}^+(GnRH_{Pit})) \cdot LH_{Pit}(t)$$

$$\frac{d}{dt} LH_{Bld}(t) = Rel_{LH}(t) - c_{LH} \cdot LH_{Bld}(t) \tag{6}$$

Follicles and corpus luteum:

$$\frac{d}{dt} Foll(t) = \widetilde{H_{11}^+}(FSH_{Bld}) - (H_{12}^+(P4) + H_{13}^+(LH_{Bld})) \cdot Foll(t) \tag{7}$$

$$\frac{d}{dt} CL(t) = SF \cdot H_{13}^+(LH_{Bld}) \cdot Foll(t) + H_{14}^+(CL) - H_{15}^+(IOF) \cdot CL(t) \tag{8}$$

Hormones produced in the ovaries:

$$\frac{d}{dt}P4(t) = c_{CL}^{P4} \cdot CL(t)^2 - c_{P4} \cdot P4(t) \tag{9}$$
$$\frac{d}{dt}E2(t) = c_{Foll}^{E2} \cdot Foll(t)^2 - c_{E2} \cdot E2(t) \tag{10}$$
$$\frac{d}{dt}Inh(t) = c_{Foll}^{Inh} \cdot Foll(t)^2 - c_{Inh} \cdot Inh(t) \tag{11}$$

Enzymes, oxytocin and inter-ovarian factors:

$$\frac{d}{dt}Enz(t) = H_{16}^{+}(P4) - c_{Enz} \cdot Enz(t) \tag{12}$$
$$\frac{d}{dt}OT(t) = H_{17}^{+}(E2) \cdot CL(t)^2 - c_{OT} \cdot OT(t) \tag{13}$$
$$\frac{d}{dt}IOF(t) = H_{18}^{+}(PGF\&CL) - c_{IOF} \cdot IOF(t) \tag{14}$$

PGF2α and synthetic prostaglandin

$$\frac{d}{dt}PGF(t) = H_{19}^{+}(Enz\&OT) - c_{PGF} \cdot PGF(t) \tag{15}$$
$$\frac{d}{dt}PGF_{syn}(t) = D \cdot \beta^2 \cdot t_{mod}(t) \cdot \exp(-\beta \cdot t_{mod}(t)) - c_{PGF_{syn}} \cdot PGF_{syn}(t)$$
$$t_{mod}(t) := \max(0, t - t_D)$$

B List of Hill functions

Positive resp. negative Hill functions are defined as

$$h^+(S(t); T, n) := \frac{S(t)^n}{T^n + S(t)^n}, \qquad h^-(S(t); T, n) := \frac{T^n}{T^n + S(t)^n}$$

The Hill functions listed below are the full notations of the Hill functions mentioned in Sections 2 and in Appendix A. They represent a majority of the

mechanisms shown in Figure 4.

$$
\begin{aligned}
H_1^-(P4\&E2) &:= m_{P4\&E2} \cdot \Big(h^-(P4(t); T_{P4}^{GnRH,1}, 2) + h^-(E2(t), T_{E2}^{GnRH,1}, 2) \\
&\qquad - h^-(P4(t); T_{P4}^{GnRH,1}, 2) \cdot h^-(E2(t), T_{E2}^{GnRH,1}, 2)\Big) \\
H_2^-(P4) &:= m_{P4}^{GnRH,2} \cdot h^-(P4(t), T_{P4}^{GnRH,2}, 2) \\
H_3^+(E2) &:= m_{E2}^{GnRH,2} \cdot h^+(E2(t), T_{E2}^{GnRH,2}, 5) \\
H_4^-(Inh) &:= m_{Inh}^{FSH} \cdot h^-(Inh(t), T_{Inh}^{FSH}, 5) \\
H_5^+(P4) &:= m_{P4}^{FSH} \cdot h^+(P4(t); T_{P4}^{FSH}, 2) \\
H_6^-(E2) &:= m_{E2}^{FSH} \cdot h^-(E2(t); T_{E2}^{FSH}, 2) \\
H_7^+(GnRH_{Pit}) &:= m_{GnRH}^{FSH} \cdot h^+(GnRH_{Pit}(t); T_{GnRH}^{FSH}, 1) \\
H_8^+(E2) &:= m_{E2}^{LH} \cdot h^+(E2(t); T_{E2}^{LH}, 2) \\
H_9^-(P4) &:= m_{P4}^{LH} \cdot h^-(P4(t); T_{P4}^{LH}, 2) \\
H_{10}^+(GnRH_{Pit}) &:= m_{GnRH}^{LH} \cdot h^+(GnRH_{Pit}(t); T_{GnRH}^{LH}, 5) \\
\widetilde{H_{11}^+}(FSH_{Bld}) &:= m_{FSH}^{Foll} \cdot h^+(FSH_{Bld}(t); \widetilde{T}_{FSH}^{Foll}(t), 2), \\
&\qquad \widetilde{T}_{FSH}^{Foll}(t) := T_{FSH}^{Foll} \cdot h^-(Foll(t); T_{Foll}^{FSH}, 2) \\
H_{12}^+(P4) &:= m_{P4}^{Foll} \cdot h^+(P4(t); T_{P4}^{Foll}, 5) \\
H_{13}^+(LH_{Bld}) &:= m_{LH}^{Ovul} \cdot h^+(LH_{Bld}(t); T_{LH}^{Ovul}, 2) \\
H_{14}^+(CL) &:= m_{CL}^{CL} \cdot h^+(CL(t), T_{CL}^{CL}, 2) \\
H_{15}^+(IOF) &:= m_{IOF}^{CL} \cdot h^+(IOF(t); T_{IOF}^{CL}, 5) \\
H_{16}^+(P4) &:= m_{P4}^{Enz} \cdot h^+(P4(t); T_{P4}^{Enz}, 5) \\
H_{17}^+(E2) &:= m_{E2}^{OT} \cdot h^+(Enz(t); T_{E2}^{OT}, 2) \\
H_{18}^+(PGF\&CL) &:= m_{PGF\&CL}^{IOF} \cdot h^+(PGF(t) + PGF_{syn}(t); T_{PGF}^{IOF}, 5) \\
&\qquad \cdot h^+(CL(t); T_{CL}^{IOF}, n_{CL}^{IOF}, 10) \\
H_{19}^+(Enz\&OT) &:= m_{Enz\&OT}^{PGF} \cdot h^+(Enz(t); T_{Enz}^{PGF}, 5) \cdot h^+(OT(t); T_{OT}^{PGF}, 2)
\end{aligned}
$$

C Parameter values

[·] stands for the unit of the substance, usually a concentration, and can be specified from measurements. Typical units are [FSH]=[LH]=IU/l, [P4]=ng/ml, and [E2]=pg/ml. If units of FSH and LH differ in pituitary and blood, release-terms have to be scaled adequately. t denotes "time"; in our model [t] stands for "days"

Parameter	Value	Unit
$GnRH_{Hypo}^{\max}$	16	$[\mathrm{GnRH_{Hypo}}]$
$c_{GnRH,1}$	2.75	$\frac{[\mathrm{GnRH_{Hypo}}]}{[\mathrm{t}]}$
$m_{P_4\&E_2}$	2.05	$1/[t]$
$T_{E_2}^{GnRH,1}$	0.0972	[E2]
$T_{P_4}^{GnRH,1}$	0.35	[P4]
$m_{P_4}^{GnRH,2}$	1.91	1/[t]
$T_{P_4}^{GnRH,2}$	0.252	[P4]
$m_{E_2}^{GnRH,2}$	0.99	$\frac{[\mathrm{GnRH_{Pit}}]}{[\mathrm{GnRH_{Hypo}}]}$
$T_{E_2}^{GnRH,2}$	0.648	[E2]
$c_{GnRH,2}$	1.63	1/[t]
m_{Inh}^{FSH}	4.21	[FSH]/[t]
T_{Inh}^{FSH}	0.118	[Inh]
b_{FSH}	0.948	1/[t]
$m_{P_4}^{FSH}$	0.293	1/[t]
$T_{P_4}^{FSH}$	0.152	[P4]
$m_{E_2}^{FSH}$	0.396	1/[t]
$T_{E_2}^{FSH}$	0.312	[E2]
m_{GnRH}^{FSH}	1.23	1/[t]
T_{GnRH}^{FSH}	0.0708	$[\mathrm{GnRH_{Pit}}]$
c_{FSH}	2.73	1/[t]
$m_{E_2}^{LH}$	0.376	[LH]/[t]
$T_{E_2}^{LH}$	0.243	[E2]
$m_{P_4}^{LH}$	2.71	[LH]/[t]
$T_{P_4}^{LH}$	0.0269	[P4]
b_{LH}	0.0141	1/[t]
m_{GnRH}^{LH}	2.22	1/[t]
T_{GnRH}^{LH}	0.69	$[\mathrm{GnRH_{Pit}}]$
c_{LH}	12.0	1/[t]
m_{FSH}^{Foll}	0.562	[Foll]/[t]
T_{FSH}^{Foll}	0.57	[FSH]
T_{Foll}^{FSH}	0.22	[Foll]
$m_{P_4}^{Foll}$	1.1	1/[t]
$T_{P_4}^{Foll}$	0.126	[P4]
m_{LH}^{Ovul}	3.49	1/[t]
T_{LH}^{Ovul}	0.171	[LH]

Parameter	Value	Unit
SF	0.2	[CL]/[t]
m_{CL}^{CL}	0.0353	[CL]/[t]
T_{CL}^{CL}	0.1	[CL]
m_{IOF}^{CL}	41.39	1/[t]
T_{IOF}^{CL}	1.32	[IOF]
c_{CL}^{P4}	2.25	$\frac{[\mathrm{P4}]/[\mathrm{CL}]^2}{[\mathrm{t}]}$
c_{P4}	1.41	1/[t]
c_{Foll}^{E2}	2.19	$\frac{[\mathrm{E2}]/[\mathrm{Foll}]^2}{[\mathrm{t}]}$
c_{E2}	1.23	1/[t]
c_{Foll}^{Inh}	1.41	$\frac{[\mathrm{Inh}]/[\mathrm{Foll}]^2}{[\mathrm{t}]}$
c_{Inh}	0.475	1/[t]
m_{P4}^{Enz}	3.58	[Enz]/[t]
T_{P4}^{Enz}	0.77	[P4]
c_{Enz}	2.98	1/[t]
m_{E2}^{OT}	1.59	$\frac{[\mathrm{OT}]/[\mathrm{CL}]^2}{[\mathrm{t}]}$
T_{E2}^{OT}	0.143	[E2]
c_{OT}	0.644	1/[t]
$m_{PGF\&CL}^{IOF}$	39.68	[IOF]/[t]
T_{PGF}^{IOF}	1.22	[PGF]
T_{CL}^{IOF}	0.6	[CL]
c_{IOF}	0.298	1/[t]
$m_{Enz\&OT}^{PGF}$	53.91	[PGF]/[t]
T_{Enz}^{PGF}	1.43	[Enz]
T_{OT}^{PGF}	1.087	[OT]
c_{PGF}	1.23	1/[t]
D	3.7	[PGF]
β	100	1/[t]
$c_{PGF_{syn}}$	5.5	1/[t]

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