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A simple mathematical model of the bovine estrous cycle: follicle development and endocrine interactions

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Abstract

Bovine fertility is the subject of extensive research in animal sciences, especially because fertility of dairy cows has declined during the last decades. The regulation of estrus is controlled by the complex interplay of various organs and hormones. Mathematical modeling of the bovine estrous cycle could help in understanding the dynamics of this complex biological system. In this paper we present a mechanistic mathematical model of the bovine estrous cycle that includes the processes of follicle and corpus luteum development and the key hormones that interact to control these processes. The model generates successive estrous cycles of 21 days, with three waves of follicle growth per cycle. The model contains 12 differential equations and 54 parameters. Focus in this paper is on development of the model, but also some simulation results are presented, showing that a set of equations and

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parameters is obtained that describes the system consistent with empirical knowledge. Even though the majority of the mechanisms that are included in the model are based on relations that in literature have only been described qualitatively (i.e. stimulation and inhibition), the output of the model is surprisingly well in line with empirical data. This model of the bovine estrous cycle could be used as a basis for more elaborate models with the ability to study effects of external manipulations and genetic differences.

Keywords:

cow, reproduction, hormone patterns, differential equations, systems biology
2000 MSC: 92C42, 92C30, 90C31, 65L09

1. Introduction

Systems biology is a relatively new research area in the field of animal sciences. It aims at understanding how the various components of a biological system function together, rather than investigating only individual parts. One approach is the translation of a conceptual biological model into a set of mathematical equations that represent the dynamic relations between system components. The purpose of building such mathematical models is to interpret and predict the dynamics of complex biological systems, and to identify new research questions.

One example of a dynamic biological system is the bovine estrous cycle, the hormonally controlled recurrent periods when the cow is preparing for reproduction by producing a fertilizable oocyte. Concurrent with selection for increased milk yield, a decrease in dairy cow fertility has been observed during the last decades (for reviews see [1, 2]). This decline in fertility is shown

15 by e.g. alterations in hormone patterns during the estrous cycle, reduced
16 expression of estrous behavior and lower conception rates [3]. However, it
17 is hard to understand which underlying mechanisms cause this decline in
18 fertility. The regulation of estrus is controlled by the interplay of various
19 organs and hormones. Mathematical modeling of the involved mechanisms is
20 expected to improve insight in the biological processes underlying the bovine
21 estrous cycle, and could thereby help to find causes of declined fertility in
22 dairy cows [4].

23 Although the endocrine and physiologic regulation of the bovine estrous
24 cycle is studied extensively, mathematical models of cycle regulation are
25 scarce and of limited scope [5, 6]. A number of models have been devel-
26 oped for other ruminant species, especially ewes [7, 8], but these models do
27 not contain all the key players that are required to simulate follicle develop-
28 ment and the accompanying hormone levels throughout consecutive cycles.
29 A model that integrates the major tissues and hormones involved, and that
30 is able to simulate the dynamics of follicular development, has been devel-
31 oped for the human menstrual cycle by Reinecke [9]. This model, which is
32 based on previous work by Selgrade and colleagues [10, 11, 12], describes the
33 dynamics of hormones, enzymes, receptors, and follicular phases throughout
34 the cycle in a set of differential equations.

35 The objective of the work described in this paper was to develop a math-
36 ematical model of the dynamics of the bovine estrous cycle on individual cow
37 level, that is able to simulate follicle development and the accompanying fluc-
38 tuations in hormone concentrations. Physiologic and endocrine mechanisms
39 that regulate the cycle are very similar between human and cows. There-

40 fore, some mechanisms of the human model in [9] could be used (although
41 sometimes with simplifications), and extended with other mechanisms like
42 follicular wave emergence and corpus luteum regression.

43 Focus in this paper is on the model development. Section 2 describes the
44 biological mechanisms of the bovine estrous cycle and how these mechanisms
45 are incorporated in the model. In Section 3, the mathematical description
46 and all model equations and parameters are given. Simulation results are
47 presented in Section 4, showing that a set of equations and parameters is
48 obtained that describes the system consistent with biological data for cows.
49 In Section 5, it is discussed how the current model could be applied and
50 extended.

51 **2. Biological background**

52 *2.1. Follicles*

53 Two different patterns of follicle development are identified in mammals.
54 In humans (and rats and pigs), the development of follicles to ovulatory
55 size occurs only during the follicular phase, while in cattle (and sheep and
56 horses), development of follicles to ovulatory or near-ovulatory size occurs
57 throughout the cycle [13]. A normal cycle includes two or three wave-like
58 patterns of follicle development, in which a cohort of follicles start to grow.
59 The average duration of the bovine estrous cycle is 20 days for 2-wave and
60 22 days for 3-wave cycles (reviewed in [14]). Each follicular wave is initi-
61 ated by an increase of follicle stimulating hormone (FSH) release from the
62 anterior pituitary [15]. The growing follicles produce estradiol (E2) and in-
63 hibin (Inh), which are released into peripheral blood. In the first one or two

64 waves, a dominant follicle deviates from the cohort of growing follicles that
65 does not ovulate, but undergoes regression under influence of progesterone
66 (P4) produced by the corpus luteum (CL). When the CL is regressed under
67 influence of $\text{PGF2}\alpha$, the concentration of P4 decreases [16]. The dominant
68 follicle present at that moment develops and matures, and ovulation can
69 then take place because the inhibiting effect of P4 on the surge of luteinizing
70 hormone (LH) is removed [17]. Elevated E2 levels increase the secretion of
71 gonadotropin releasing hormone (GnRH), which triggers the LH surge and
72 thereby induces ovulation. Once an oocyte is successfully ovulated, the re-
73 mains of the follicle form a new P4-producing CL. If conception has failed,
74 the CL regresses, P4 levels decrease, and the cycle restarts (reviewed in [4]).

75 The ovaries contain a pool of small follicles with immature oocytes. Un-
76 der influence of FSH, a cohort of 8-41 growing follicles emerge [14]. Approx-
77 imately two days after cohort recruitment, one follicle is selected to become
78 the dominant follicle, and continues to grow [18]. This deviation of the dom-
79 inant follicle is associated with increased FSH and LH receptor binding, ac-
80 tivating the enzymes that catalyze steroidogenesis, resulting in increased E2
81 production and higher E2 serum levels [18]. The dominant follicle expresses
82 more FSH receptors, and it can therefore continue to grow even when FSH
83 serum levels are low [19]. In the model, the emergence of a follicular wave
84 is induced when FSH exceeds a threshold which becomes lower when fol-
85 licles become larger, representing that larger follicles are more sensitive to
86 FSH. Dominant follicles also secrete increasing amounts of inhibin (Inh). Inh
87 suppresses FSH and, hence, suppresses the growth of subordinate follicles.
88 Ovulation or regression of the dominant follicle eliminates this suppression,

89 allowing the onset of the next follicular wave [20, 21].

90 Small follicles of an emerging cohort may release very small amounts of
91 E2 and Inh per follicle, but taken together, this amount is not negligible. Fur-
92 thermore, there is always a medium-size or large follicle present [22, 23, 24],
93 which results in a basal hormone production throughout the cycle. Differ-
94 ent follicles are recruited, growing, and regressing in each cycle and in each
95 wave. However, total E2 and Inh production capacity is modeled as a contin-
96 uous function throughout subsequent waves and cycles, representing the total
97 amount of hormone production of the follicles present at any moment. Folli-
98 cle regression is promoted by high P4 levels and by the LH surge (Equation
99 7). The capacity of follicles to produce E2 and Inh is denoted as “follicular
100 function” in the rest of this paper.

101 2.2. *Corpus luteum*

102 The CL develops within 2-3 days after ovulation, starting the synthesis
103 and release of P4, which maintains the readiness of the endometrium for
104 receiving the embryo. In absence of a conceptus, the CL will regress at day 17-
105 18 of the cycle [25, 26]. In each cycle a new CL develops, but CL development
106 is modeled as a continuous function of P4 producing tissue, denoted as “CL
107 function” in the rest of this paper. In the model, CL development is induced
108 by the LH surge. A threshold and delay are incorporated in the effect of LH
109 on the CL, to account for the time required for the process of transition from
110 follicle to CL [16] and the shift from E2 to P4 production [27, 28]. If the CL
111 reaches a certain size, it continues to grow without further stimulation by LH
112 [29]. CL regression is induced by PGF2 α secretion from the uterus (described
113 in Section 2.4). Growth and regression of CL function are described by

114 Equation 9.

115 *2.3. Estradiol and inhibin*

116 E2 affects LH synthesis and release [30] and FSH release [19, 31]. E2
117 serum levels are higher in ovulatory than in non-ovulatory waves [20, 32] and
118 reach peak levels around estrus [20, 32, 33, 34, 35, 23]. This suggests that
119 the preovulatory follicle has the largest capacity to produce and release E2,
120 although its maximum size is not significantly different from the maximum
121 size of non-ovulatory dominant follicles. Considering the results in [36, 37],
122 where a better vascularity of the ovulatory follicle is reported, it is reasonable
123 that the ovulatory follicle can secrete more E2 than non-ovulatory follicles
124 and, consequently, E2 serum levels are highest at estrus. In the model, the
125 rate of E2 production and release to the blood is taken as proportional to
126 follicular function (Equation 11).

127 Inh inhibits FSH synthesis and thus reduces FSH release [21]. Compared
128 to basal Inh serum levels, peak levels are almost doubled in non-ovulatory
129 waves and increase further in ovulatory waves [38]. There are different forms
130 of inhibin, but only inhibin A is considered in the model, as it is the predom-
131 inant form in bovine follicular fluid [19]. In the model, Inh production rate
132 is taken as proportional to follicular function (Equation 12).

133 *2.4. Progesterone and prostaglandin $F2\alpha$*

134 The CL is the main source of P4. Serum P4 concentration is near to zero
135 around estrus and high during the luteal phase [39, 40, 32, 41, 42]. A high
136 correlation between CL diameter and P4 output was reported in [43, 44, 24].

137 In the model, the rate of P4 release into the blood is taken as proportional
138 to CL function (Equation 10).

139 Pulsatile PGF2 α release from the uterus induces CL regression. The
140 rise of P4 early in the cycle initiates a series of events or mechanisms that
141 eventually lead to the rise of PGF2 α , followed by a decline of PGF2 α a
142 few days later. It was shown that administration of P4 prior to its natural
143 rise resulted in an equally earlier onset of CL regression [45]. Exposure to
144 effective amounts of P4 must last for 10-13 days to induce PGF2 α pulses
145 [45, 46, 47, 48]. Peak PGF2 α levels are 3-4 times higher than basal levels
146 [49, 50, 51, 52].

147 PGF2 α is regulated by oxytocin (OT), P4 and E2 [53]. P4 first prevents
148 a too early release of PGF2 α pulses, but simultaneously stimulates synthe-
149 sis of enzymes required for PGF2 α production. In the later luteal phase,
150 changed expression of P4 and OT receptors results in a gradual decrease in
151 the suppression of PGF2 α [49], leading to an OT induced pulsatile release
152 of PGF2 α [52, 46]. How these mechanisms are regulating each other is quite
153 complex and not understood in full detail.

154 What is clear is that the rise in P4 levels and the continued presence of
155 P4 above an effective level sets in motion a series of events that lead to CL
156 regression. Hence, we incorporated these series of events as a black box using
157 time delays to obtain the right timing of PGF2 α signaling. In the model,
158 PGF2 α increases a specific number of days (delay $\tau_{P4,1}$) after P4 levels reach a
159 threshold. Similarly, PGF2 α declines another (larger) number of days (delay
160 $\tau_{P4,2}$) after P4 levels reached a threshold (Equation 8).

161 *2.5. Gonadotropin releasing hormone, luteinizing hormone and follicle stim-*
162 *ulating hormone*

163 Pulsatile signaling of GnRH regulates LH and FSH secretion [54]. Because
164 GnRH induces the LH surge, it indirectly induces ovulation [55]. The GnRH
165 pulse generator is located in the hypothalamus and is modulated by P4 and
166 E2 [56]. During the luteal phase, both P4 and E2 suppress the activity of the
167 GnRH pulse generator. During pro-estrus however, elevated E2 levels change
168 estrogen receptor signaling, which induces a GnRH surge [30, 56]. GnRH
169 is released into the portal circulation of the pituitary and binds to GnRH
170 receptors of the anterior pituitary [57]. In the model, GnRH stimulates
171 LH release, resulting in an LH surge concurrently with the GnRH surge.
172 GnRH synthesis is taken constant as long as the amount of GnRH in the
173 hypothalamus is below a threshold (Equation 1). GnRH release is inhibited
174 when P4 levels are above a threshold and when both P4 and E2 levels are
175 above a threshold. GnRH release is stimulated when P4 levels are low and
176 E2 reaches a threshold (Equation 1b), resulting in a surge of GnRH. GnRH
177 concentration in the pituitary depends on GnRH amount released from the
178 hypothalamus, and is further increased by high E2 levels, representing that
179 E2 up-regulates expression of GnRH receptors [56, 57] (Equation 2).

180 The LH surge at the day before ovulation induces ovulation of the ovu-
181 latory follicle and formation of the CL. The LH surge will shut down E2
182 and Inh production capacity of the ovulatory follicle [58, 24]. High P4 levels
183 suppress the release of LH via the inhibition of the GnRH pulse generator
184 [59]. Additionally, high P4 levels decrease pituitary sensitivity to E2, thereby
185 increasing the amount of E2 required to induce an LH surge above physio-

186 logical levels [56]. Peak LH levels are about five times as high as basal levels
187 or higher [20, 32, 60, 27]. In the model, LH synthesis is stimulated by E2
188 and inhibited by P4 (Equation 5a). Besides a small basal LH release, there
189 is a surge of LH when GnRH in the pituitary reaches a threshold (Equation
190 5b).

191 FSH synthesis is inhibited by Inh [19]. P4 and E2 modulate FSH release
192 via effects on the anterior pituitary and on the GnRH pulse generator in
193 the hypothalamus. Peak FSH serum levels are about three times higher than
194 basal levels [20, 33]. In the model, FSH synthesis in the pituitary is increased
195 when Inh levels are below a threshold (Equation 3a). FSH release from the
196 pituitary to the blood is stimulated by P4 and GnRH, and inhibited by E2
197 (Equation 3b).

198 **3. Mathematical formulation**

199 The mathematical approach used for the bovine model is comparable
200 to the approach used for the model of the human menstrual cycle, which
201 originally has been developed at North Carolina State University by Selgrade
202 and colleagues [10, 11, 61, 12], and has been extended at the Zuse Institute
203 [9, 62].

204 The system is considered in four compartments: hypothalamus, anterior
205 pituitary, ovaries and uterus, connected through peripheral and portal blood
206 (Figure 1). The model includes the processes of follicle and CL development
207 and the key hormones that interact to control these processes as described
208 in Section 2. The gonadotropin equations are based on synthesis-release-
209 clearance relations. This structure was first introduced in [11]. The complete

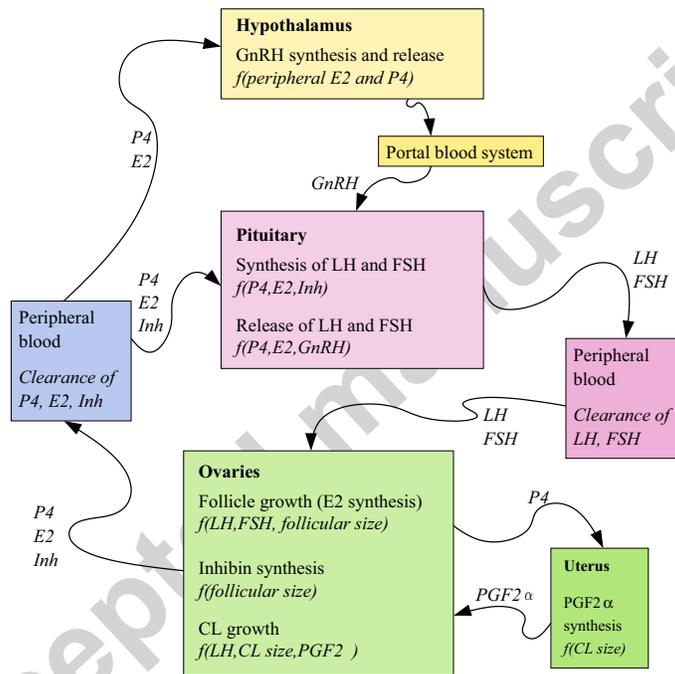


Figure 1: Schematic representation of the compartments in the model of the bovine estrous cycle.

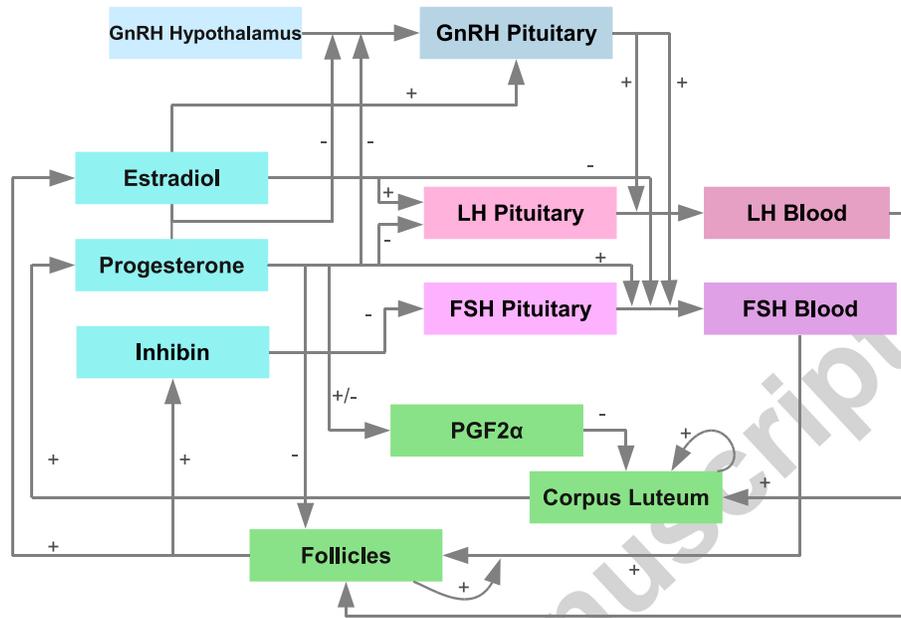


Figure 2: Complete mechanisms of the bovine model. Boxes represent the 12 key components of the system. Differential equations are derived for these 12 components. Arrows denote functional dependencies. Stimulating and inhibiting effects are indicated by + and - respectively.

210 mechanisms are shown in Figure 2.

211 Based on these mechanisms, 12 ordinary differential equations (ODEs)
 212 with 54 parameters are formulated. If necessary, time delays are incorporated
 213 to model the time between events and their effects, representing the duration
 214 of intermediate steps in biological processes. In this case, the ODE is turned
 215 into a delay differential equation (DDE). To solve the system of differential
 216 equations, we use the solver RADAR5 [63], which has been designed for the
 217 solution of stiff delay differential equations.

218 *3.1. Hill functions*

219 Because the exact mechanisms are often not known or more specific than
 220 necessary, Hill functions are used to model stimulatory and inhibitory effects
 221 of the hormones. They are used whenever there is a nonlinear relation be-
 222 tween two substances. A Hill function is a sigmoidal function between zero
 223 and one, which switches at a specified threshold from one level to the other
 224 with a specified steepness. Positive Hill functions are used for stimulating
 225 effects and are defined as

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

226 $S(t)$ represents the effector, T the threshold for change of behavior, and
 227 n controls the steepness of the curve. Negative Hill functions are used for
 228 inhibitory effects and are defined as

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

229 Here, the value of the function has its maximum at the lowest value of the
 230 initiating substrate $S(t)$, and switches to zero if this substrate passes the
 231 threshold T .

232 Whenever a Hill function is used, it is provided with another parameter
 233 m that controls the height of the switch. This parameter serves as maximum
 234 stimulatory respectively inhibitory effect. For abbreviation of notation, we
 235 use $H^+(S)$ instead of $m \cdot h^+(S(t); T, n)$. We usually choose the steepness
 236 coefficient $n = 2$, but, when appropriate, we set $n = 1, 5$, or 10 to capture
 237 smoother or steeper effects. The complete set of Hill functions is specified in
 238 Appendix A, and parameter values can be found in Appendix B.

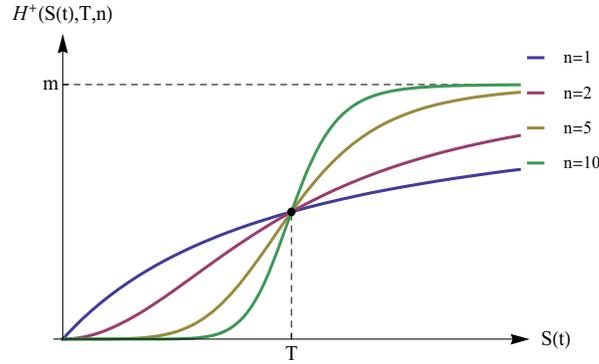


Figure 3: Scaled positive Hill functions with different steepness.

239 3.2. Model equations

240 The amount of GnRH in the hypothalamus is a result of synthesis in the
 241 hypothalamus and release into the pituitary,

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

242 GnRH synthesis depends on its current level in the hypothalamus. If this
 243 level approaches a specified threshold, synthesis decreases to zero. This effect
 244 is modeled as

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

245 As long as GnRH is far below its maximum, the factor $1 - \frac{GnRH_{Hypo}(t)}{GnRH_{Hypo}^{max}}$ has
 246 only a small impact. The release of GnRH from the hypothalamus to the
 247 pituitary is dependent on its current level in the hypothalamus. E2 inhibits
 248 GnRH release during the luteal phase, i.e. if P4 and E2 are high at the
 249 same time, described by $H_1^-(P4 \& E2)$. $H_1^-(P4 \& E2)$ denotes the sum of two
 250 Hill functions minus their product, and inhibits GnRH release only if both

251 substrates are above their threshold. Additionally, the release of GnRH is
 252 inhibited by P4 only,

$$Rel_{GnRH}(t) = (H_1^-(P4 \& E2) + H_2^-(P4)) \cdot GnRH_{Hypo}(t). \quad (1b)$$

253 Changes in GnRH amount in the pituitary are dependent on the released
 254 amount from the hypothalamus, but also on the presence of E2. E2 increases
 255 the number of GnRH receptors in the pituitary. This effect is included in the
 256 equation as a positive Hill function. GnRH clearance from pituitary portal
 257 blood is proportional to the GnRH level in the pituitary, i.e. GnRH clearance
 258 is represented by $c_{GnRH,2} \cdot GnRH_{Pit}(t)$, in which $c_{GnRH,2}$ is a constant,

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

259 FSH is synthesized in the pituitary and released into the blood,

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

260 FSH synthesis rate in the pituitary is only dependent on delayed Inh, as in
 261 [61]. FSH is synthesized when the Inh level is low, i.e. high Inh levels inhibit
 262 FSH synthesis, which is included as a negative Hill function,

$$Syn_{FSH} = H_4^-(Inh_\tau). \quad (3a)$$

263 The index τ stands for a delayed effect of Inh, i.e. Inh is considered at time
 264 $t - \tau$. FSH release from the pituitary to the blood is stimulated by P4 and
 265 GnRH, and inhibited by E2,

$$Rel_{FSH} = (H_5^+(P4) + H_6^-(E2) + H_7^+(GnRH_{Pit})) \cdot FSH_{Pit}(t). \quad (3b)$$

266 Concluding, FSH serum level is a result of the difference between the released
267 amount from the pituitary and clearance in the blood,

$$\frac{d}{dt}FSH_{Blood}(t) = Rel_{FSH}(t) - c_{FSH} \cdot FSH_{Blood}(t), \quad (4)$$

268 where c_{FSH} is the FSH clearance rate constant.

269 Like FSH, the LH serum level depends on synthesis in the pituitary, re-
270 lease into the blood and clearance thereof,

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

271 LH synthesis in the pituitary is stimulated by E2 and inhibited by P4,

$$Syn_{LH}(t) = H_8^+(E2) + H_9^-(P4). \quad (5a)$$

272 We assume a low constant basal LH release b_{LH} from the pituitary into the
273 blood. On top of that, LH release is stimulated by GnRH,

$$Rel_{LH}(t) = (b_{LH} + H_{10}^+(GnRH_{Pit})) \cdot LH_{Pit}(t). \quad (5b)$$

274 Summarizing, LH in the blood is obtained as

$$\frac{d}{dt}LH_{Blood}(t) = Rel_{LH}(t) - c_{LH} \cdot LH_{Blood}(t), \quad (6)$$

275 where c_{LH} is the LH clearance rate constant.

276 Follicular function is stimulated by FSH, whereas its decrease is promoted
277 by P4 and the LH surge,

$$\frac{d}{dt}Foll(t) = H_{11}^+(FSH) - (H_{12}^+(P4) + H_{13}^+(LH_{Blood})) \cdot Foll(t). \quad (7)$$

The sensitivity of the follicles to respond to FSH grows with their size. In the model, the threshold of FSH to stimulate the follicular function decreases

with increasing follicular function. For this effect of a rising FSH sensitivity, a negative Hill function is included to control the threshold of FSH,

$$\tilde{T}_{FSH}^{Foll}(t) := T_{FSH}^{Foll} \cdot h^-(Foll(t); T_{Foll}^{FSH}, 1),$$

278 and the Hill function for the effect of FSH on follicular function becomes

$$H_{11}^+(FSH) := m_{FSH}^{Foll} \cdot h^+(FSH_{Blood}(t); \tilde{T}_{FSH}^{Foll}(t), 2). \quad (7a)$$

279 PGF2 α initiates the functional regression of the CL, and thereby the
280 decrease in P4 levels. After a large time delay, PGF2 α synthesis is stimulated
281 by elevated P4 levels above a specified threshold value. The PGF2 α level
282 declines a couple of days after its rise, which is included as a delayed positive
283 effect of P4 on the decay of PGF2 α ,

$$\frac{d}{dt}PGF2\alpha(t) = H_{14}^+(P4_{\tau_1}) - H_{15}^+(P4_{\tau_2}) \cdot PGF2\alpha(t). \quad (8)$$

284 The LH peak initiates growth of the CL with a specified delay. After
285 reaching a certain size, the CL continues to grow on its own as long as
286 PGF2 α is low. The CL starts to regress when PGF2 α levels rise above a
287 threshold,

$$\frac{d}{dt}CL(t) = H_{16}^+(LH_{\tau}) + H_{17}^+(CL) - H_{18}^+(PGF2\alpha) \cdot CL(t). \quad (9)$$

288 The production of P4 in the ovary is assumed to be proportional to CL
289 function, and the production of E2 and Inh is assumed to be proportional
290 to follicular function. Therefore, the equations for P4, E2, and Inh do not

291 contain any Hill functions,

$$\frac{d}{dt}P_4(t) = c_{CL}^{P_4} \cdot CL(t) - c_{P_4} \cdot P_4(t), \quad (10)$$

$$\frac{d}{dt}E_2(t) = c_{Foll}^{E_2} \cdot Foll(t) - c_{E_2} \cdot E_2(t), \quad (11)$$

$$\frac{d}{dt}Inh(t) = c_{Foll}^{Inh} \cdot Foll(t) - c_{Inh} \cdot Inh(t). \quad (12)$$

292 The parameters c_{P_4} , c_{E_2} and c_{Inh} denote the respective clearance rate con-
293 stants.

294 Figure 2 gives an overview of all mechanisms described by the model equa-
295 tions. Detailed notations for the Hill functions, parameters, and equations
296 are given in Appendix A, Appendix B, and Appendix C respectively.

297 3.3. Parameter identification and sensitivity analysis

298 The main difficulty is not to simulate the system, i.e. to solve the dif-
299 ferential equations, but to identify the unknown parameters. Unfortunately,
300 many of the parameters are not measurable. Sometimes the range of values
301 is known, but some parameters are completely unknown. The techniques
302 for parameter estimation that are used in this model are implemented in
303 the software packages PARKIN [64, 65] and NLSCON [66], which have been
304 developed at the Zuse Institute for many years. These programs take into ac-
305 count parameter sensitivities and linear dependencies, and include a number
306 of optimization methods such as, for example, affine covariant Gauss-Newton
307 methods [67]. A renewed version of this software, especially adapted to pa-
308 rameter identification in ordinary differential equation models, has been used
309 throughout the paper. The mathematical background is described in [67].

310 To obtain a good initial guess for the parameter optimization procedure,
311 we use a model decomposition approach and successively enlarge the set of

312 estimated parameters. The first step is to define input curves representing
 313 the development of Inh, P4, and E2 levels in the blood over time. This use
 314 of explicit functions, which simplifies parameter identification, was already
 315 suggested by Schlosser [11]. Composition of these input curves is based on
 316 published data for endocrine profiles of cows with a normal estrous cycle, see
 317 for example [68].

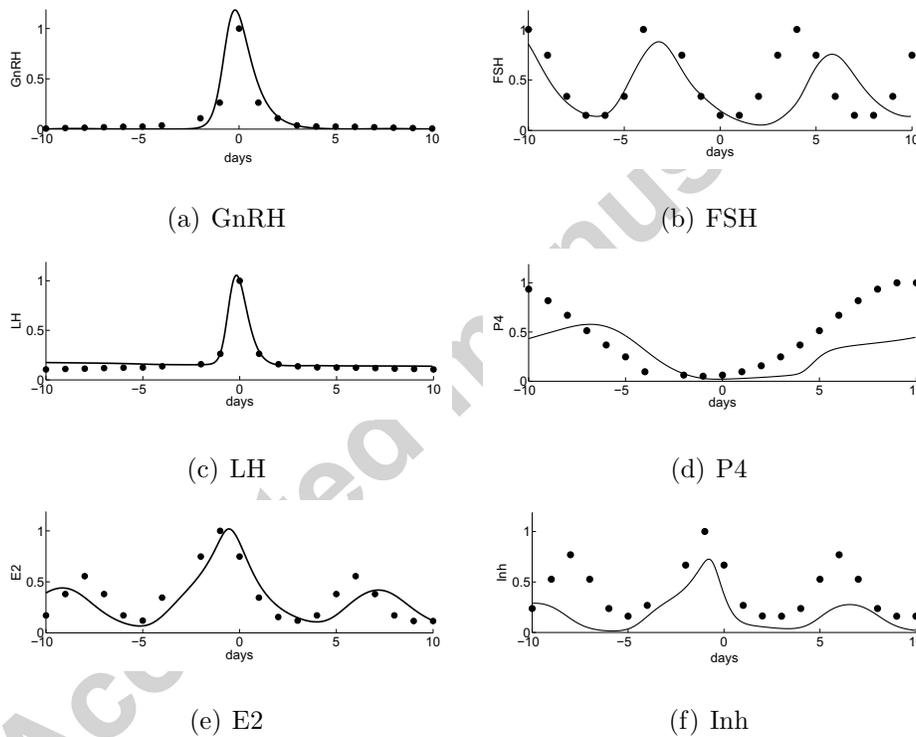


Figure 4: Simulated curves of the closed model together with the data points used for parameter estimation. Panels 4(a), 4(b) and 4(c) show data points based on qualitative behavior of hormones as described in literature ([68]). Panels 4(d), 4(e) and 4(f) show data points obtained from the input curves. Day zero corresponds to the day of LH peak.

318 Following the approach in [61], we use the input curves to successively fit
 319 the profiles of the other components. The detailed procedure can be found

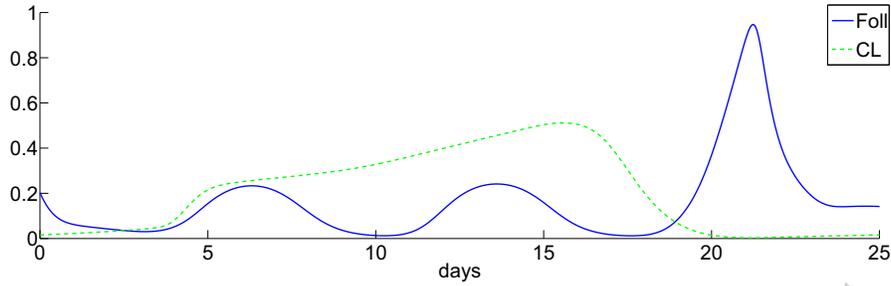
320 in [69]. In the last step, the input curves for P4, E2, and Inh are replaced by
 321 their original ODE/DDE description to obtain a closed network. The final
 322 parameter values are listed in Table B.1, and the corresponding simulation
 323 results are illustrated in Figure 4.

324 A sensitivity analysis has been performed with the techniques described in
 325 [67]. A more detailed description including column norms of the sensitivity
 326 matrix and subconditions, which provide information about the sensitivities
 327 and the dependencies of the parameters, can be found in [69]. It turns out
 328 that among the most sensitive and best predictable parameters are $p_{36} =$
 329 $\tau_{P4,1}$, $p_{11} = \tau_{Inh}$, $p_{20} = c_{FSH}$, and $p_{39} = \tau_{P4,2}$.

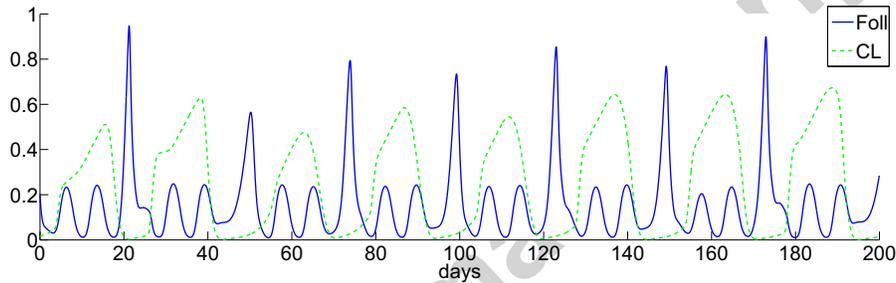
330 4. Simulation results

331 The figures in this section show the computed dynamics of follicle and
 332 CL development and accompanying fluctuations in hormone levels over con-
 333 secutive cycles. The simulation results show that the current set of model
 334 parameters generates curves consistent with empirical knowledge for cows
 335 with a normal estrous cycle with three follicular waves. Notice that the model
 336 generates consecutive cycles that are not entirely identical (quasi-periodic be-
 337 havior), but that vary slightly in patterns and peak heights between cycles.
 338 Small differences in model output at the end of a cycle result in a different
 339 starting point of the next cycle, which leads to variation between the curves.
 340 This variation in hormone levels between cycles could well resemble variation
 341 within a cow over consecutive cycles. However, a different parameterization
 342 can be used to produce a stable limit cycle.

343 Each estrous cycle contains three waves of follicular growth (Figure 5).



(a)

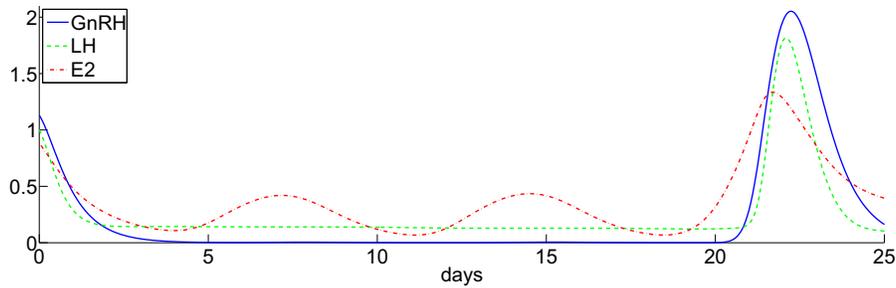


(b)

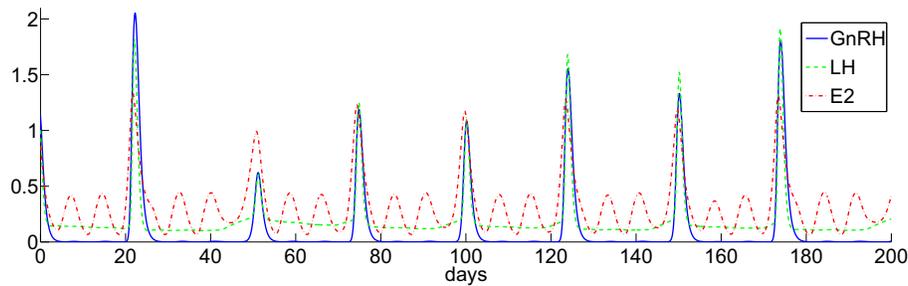
Figure 5: Output curves of follicular function (Foll) and CL function (CL) over time for one cycle (a) and in consecutive cycles (b).

344 The CL starts to grow a few days after ovulation and is large during the first
 345 two follicular waves, which suppresses follicle growth. As the larger follicles
 346 become more sensitive to P4, at a certain size the effect of P4 becomes so large
 347 that it induces follicle regression. After regression of the CL, the dominant
 348 follicle of the third follicular wave can continue to grow, leading to ovulation,
 349 which causes a sharp decline in follicular function.

350 The pattern of serum E2 levels is a result of follicular function (Figures
 351 5 and 6). The third wave of follicular growth takes place when P4 levels



(a)

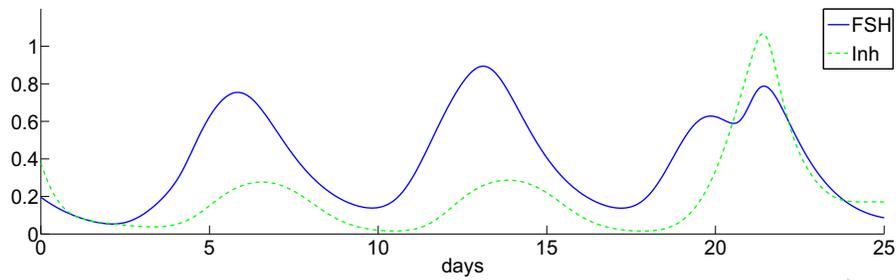


(b)

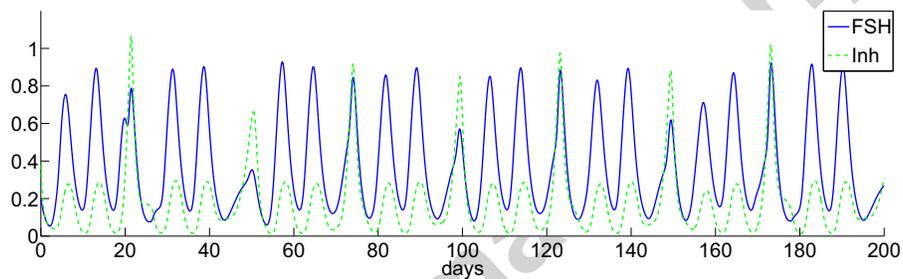
Figure 6: Output curves of serum concentrations of E2 and LH, and portal concentration of GnRH over time for one cycle (a) and in consecutive cycles (b).

352 are low, resulting in increased E2 levels. These increased E2 levels induce a
 353 steep GnRH and LH surge, which is the trigger for ovulation. Notice that
 354 the height of the GnRH surge is determined by the E2 peak level. During the
 355 remaining cycle, GnRH and LH levels are low, representing the lower pulse
 356 frequency and amplitude compared to the surge.

357 Increased FSH levels induce the growth of a follicular wave and thereby
 358 the start of Inh increase, but FSH is suppressed when Inh levels are above
 359 a certain level (Figure 7). Notice that FSH peak levels in the third wave of



(a)

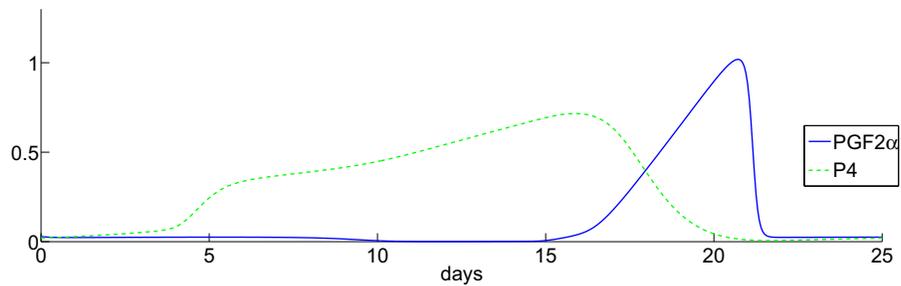


(b)

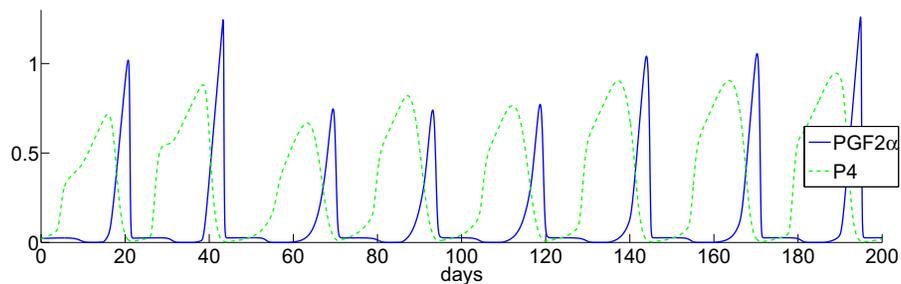
Figure 7: Output curves of serum concentrations of Inh and FSH over time for one cycle (a) and in consecutive cycles (b).

360 the cycle differ in consecutive cycles because of corresponding differences in
 361 height of the GnRH surge (Figures 6 and 7). When Inh has declined due
 362 to follicular regression, FSH increases again and induces the next follicular
 363 wave. Because follicular growth is modeled in three waves, also Inh levels
 364 rise in three waves in a cycle.

365 P4 serum levels are proportional to CL function. P4 concentration is
 366 small during the first days of the cycle and rises when the CL starts to
 367 grow (Figure 5). Notice that a lower LH peak height results in a less steep



(a)



(b)

Figure 8: Output curves of serum concentrations of P4 and PGF2 α over time for one cycle (a) and in consecutive cycles (b).

368 P4 increase and lower levels of P4 in the following cycle (Figures 6 and 8).
 369 Increased P4 levels induce a rise in PGF2 α after a couple of days, which
 370 causes CL regression and declining P4.

371 5. Discussion and Outlook

372 The current mathematical model describes the interaction between a
 373 number of key physiological processes of the bovine estrous cycle. The model
 374 is able to simulate the dynamics of follicle and CL growth and development,

375 as well as the associated hormone level changes in consecutive cycles. The
376 current model comprises 12 equations and 54 parameters. The estrous cycles
377 generated by the model are not entirely identical and could well resemble
378 variations within a cow over consecutive cycles.

379 The above simulations show a quasi-periodic behavior, but a different
380 parameterization (not listed in this paper) could be used to produce a stable
381 limit cycle. This shows that the variations between simulated cycles are not
382 an intrinsic characteristic of the model, but depend on the parameterization.
383 However, the cycles of a real cow are usually quite irregular, and we think
384 this is not due to changes in external factors for that cow but rather arises
385 from the fact that each cycle presents slightly new and somewhat different
386 ‘starting values’ for the next cycle, which we think that our model mimics.
387 Alternatively, one could add a stochastic component to the regular system
388 (representing small variations in external factors) to induce variations in
389 consecutive cycles, but this was not in the scope of our work.

390 The sensitivity analysis shows that parameters 36, 20 and 11 are the
391 most sensitive parameters of the model, which means that a small change
392 in the value of one of these parameters will have a large effect on the model
393 solution. Parameter 36 (delay of P4 until stimulating $\text{PGF}_{2\alpha}$ increase) is
394 possibly a sensitive parameter because CL life span is critical for the duration
395 of the cycle. Parameter 20 (FSH clearance rate constant) and Parameter 11
396 (delay of Inh in FSH synthesis) are possibly sensitive parameters because
397 FSH and Inh serum levels have an important effect on the progress of follicle
398 development.

399 The modeling method with ODEs/DDEs as used for the presented model

400 of the bovine estrous cycle was also used for the model of the human men-
401 strual cycle [10, 11, 61, 9]. As we aimed at the development of a model
402 for the dynamical changes of a biological system, including the information
403 about how components influence the rates of change of other components,
404 our approach to model the system with differential equations appears to be
405 the most reasonable. Maybe qualitative results could have been obtained
406 with other methods such as, for example, boolean networks, but differen-
407 tial equations allow for a simulation of quantitative profiles of the involved
408 components. To our knowledge, no comparable models of the bovine estrous
409 cycle are available.

410 The current model describes the mechanisms of an idealized cow, based
411 on average numbers obtained from several data sources. It would in principle
412 be possible to fit the model to measurement data of an individual cow that
413 would show small deviations of the cycle, or even a pathological abnormal
414 cycle due to certain disorders. This would represent the next step in the
415 modeling approach. Because empirical data are usually noisy, parameter
416 optimization would then also have to take measurement errors into account.

417 Although the current model could thus offer possibilities to simulate fer-
418 tility disorders, its predictive ability may be limited in those parts and for
419 those aspects in which the model is not entirely mechanistic but rather de-
420 scriptive. One example thereof is the modeling of $\text{PGF}2\alpha$. Because the
421 detailed biological mechanisms that induce the rise of $\text{PGF}2\alpha$ are very com-
422 plex and not completely understood, we chose to restrict the number of state
423 variables for this part of the model, and to include time delays. This mimics
424 the situation in cows that the rise of P4 early in the cycle starts a series of

425 events or mechanisms that eventually lead to the rise of $\text{PGF2}\alpha$, followed by
426 a decline of $\text{PGF2}\alpha$ several days later. The time delays are thus a ‘black box’
427 where the intermediate events that regulate $\text{PGF2}\alpha$ levels are not described.
428 In this way, we were able to obtain the right time point of CL regression even
429 though we don’t know the biological mechanisms exactly. By reducing the
430 delays, the duration of the luteal phase can be reduced. This could mean
431 that P4 serum levels already decline during the second wave of follicle devel-
432 opment, which could then become the ovulatory wave. The shorter delays
433 could thus result in a shorter cycle with only two follicular waves. However,
434 the consequence of the chosen approach is that the predictive abilities for
435 this part of the model are limited. Model improvement and refinement of
436 this sub-model will play an important role in future work.

437 Apart from fitting of the model to individual cow data, mentioned above,
438 we plan to use this model to determine the level of control exerted by vari-
439 ous system components on the functioning of the system. Examples of such
440 model applications are to explore the mechanisms that influence the pattern
441 of follicular waves, or to study hormone patterns associated with subfertility.
442 Also, the model can serve as a basis for more elaborate models and simula-
443 tions, with the ability to study effects of external manipulations and genetic
444 differences. Possible extensions of the model could be in the field of energy
445 metabolism, stress, disease, and factors affecting the expression of estrous be-
446 havior. There are relationships between regulation of the estrous cycle and
447 energy balance, which can cause fertility problems in high producing dairy
448 cows in negative energy balance (for reviews see [70, 71]). Changes in repro-
449 ductive performance that are associated with high milk production may in

450 part be explained by elevated P4 and E2 clearance rates, as described in the
451 physiological model of [3]. In this physiological model, clearance rates of hor-
452 mones by the liver of cows with high milk production are increased as a result
453 of elevated feed intake, leading to an increased liver blood flow and metabolic
454 activity. With a similar level of hormone production, circulating hormone
455 levels would thus be lower. Lameness, an example of a stress inducing condi-
456 tion, was found to inhibit the LH surge and ovulation, whereas incidence of
457 estrous behavior (although with less intensity) was not reduced. These obser-
458 vations suggest that stress, caused by lameness, reduces P4 exposure before
459 estrus and/or E2 production by the dominant follicle [72, 73]. Further, a
460 normal endocrinological cycle is prerequisite for appropriate expression of
461 estrous behavior. The relationships found between P4, E2 and intensity of
462 estrous behavior show that hormones involved in regulation of the estrous
463 cycle also affect the expression of estrous behavior [74, 75]. These and other
464 findings and hypotheses about regulation of the bovine estrous cycle could
465 be translated into mathematical equations or modified parameterization and
466 incorporated in the current model.

467 **Acknowledgements**

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474 **Appendix A. List of Hill functions**

The Hill functions listed below are the full notations of the Hill functions mentioned in Section 3.2 and represent the mechanisms shown in Figure 2.

$$\begin{aligned}
H_1^-(P_4 \& E_2) &:= m_{P_4 \& E_2} \cdot \left(h^-(P_4(t); T_{P_4}^{GnRH,1}, 2) + h^-(E_2(t), T_{E_2}^{GnRH,1}, 2) \right. \\
&\quad \left. - h^-(P_4(t); T_{P_4}^{GnRH,1}, 2) \cdot h^-(E_2(t), T_{E_2}^{GnRH,1}, 2) \right) \\
H_2^-(P_4) &:= m_{P_4}^{GnRH,2} \cdot h^-(P_4(t), T_{P_4}^{GnRH,2}, 2) \\
H_3^+(E_2) &:= m_{E_2}^{GnRH,2} \cdot h^+(E_2(t), T_{E_2}^{GnRH,2}, 5) \\
H_4^-(Inh_\tau) &:= m_{Inh}^{FSH} \cdot h^-(Inh(t - \tau_{Inh}), T_{Inh}^{FSH}, 2) \\
H_5^+(P_4) &:= m_{P_4}^{FSH} \cdot h^+(P_4(t); T_{P_4}^{FSH}, 2) \\
H_6^-(E_2) &:= m_{E_2}^{FSH} \cdot h^-(E_2(t); T_{E_2}^{FSH}, 2) \\
H_7^+(GnRH_{Pit}) &:= m_{GnRH}^{FSH} \cdot h^-(GnRH_{Pit}(t); T_{GnRH}^{FSH}, 1) \\
H_8^+(E_2) &:= m_{E_2}^{LH} \cdot h^+(E_2(t); T_{E_2}^{LH}, 2) \\
H_9^-(P_4) &:= m_{P_4}^{LH} \cdot h^-(P_4(t); T_{P_4}^{LH}, 2) \\
H_{10}^+(GnRH_{Pit}) &:= m_{GnRH}^{LH} \cdot h^+(GnRH_{Pit}(t); T_{GnRH}^{LH}, 2) \\
H_{11}^+(FSH) &:= m_{FSH}^{Foll} \cdot h^+(FSH_{Blood}(t); \tilde{T}_{FSH}^{Foll}(t), 2), \\
\tilde{T}_{FSH}^{Foll}(t) &:= T_{FSH}^{Foll} \cdot h^-(Foll(t); T_{Foll}^{FSH}, 1) \\
H_{12}^+(P_4) &:= m_{P_4}^{Foll} \cdot h^+(P_4(t); T_{P_4}^{Foll}, 2) \\
H_{13}^+(LH) &:= m_{LH}^{Foll} \cdot h^-(LH_{Blood}(t); T_{LH}^{Foll}, 2) \\
H_{14}^+(P_4)_\tau &:= m_{P_4}^{PGF2\alpha,1} \cdot h^+(P_4(t - \tau_{P_4,1}), T_{P_4}^{PGF2\alpha}, 2) \\
H_{15}^+(P_4)_\tau &:= m_{P_4}^{PGF2\alpha,2} \cdot h^+(P_4(t - \tau_{P_4,2}), T_{P_4}^{PGF2\alpha}, 10) \\
H_{16}^+(LH)_\tau &:= m_{LH}^{CL} \cdot h^+(LH_{Blood}(t - \tau_{LH}); T_{LH}^{CL}, 2) \\
H_{17}^+(CL) &:= m_{CL}^{CL} \cdot h^+(CL(t); T_{CL}^{CL}, 2) \\
H_{18}^+(PGF2\alpha) &:= m_{PGF2\alpha}^{CL} \cdot h^+(PGF2\alpha(t); T_{PGF2\alpha}^{CL}, 1)
\end{aligned}$$

475 **Appendix B. List of parameters**

476 In our model, $[\cdot]$ stands for the unit of the substance, usually a con-
 477 centration, and can be specified from measurements. Typical units are
 478 $[\text{FSH}] = [\text{LH}] = \text{IU/l}$, $[\text{P4}] = \text{ng/ml}$, and $[\text{E2}] = \text{pg/ml}$. t denotes “time”; in our
 479 model $[t]$ stands for “days”.

Table B.1: List of parameters.

No.	Symbol	Value	Quantity	Explanation
1	$GnRH_{Hypo}^{\max}$	20	$[\text{GnRH}_{\text{Hypo}}]$	maximum value for GnRH in the hypothalamus
2	$c_{GnRH,1}$	4.657	$\frac{[\text{GnRH}_{\text{Hypo}}]}{[t]}$	synthesis rate constant of GnRH in the hypothalamus
3	$m_{P4\&E2}$	1.464	$\frac{[\text{GnRH}_{\text{Hypo}}]}{[t]}$	maximum part of GnRH synthesis rate constant inhibited by E2 and P4
4	$T_{E2}^{GnRH,1}$	0.1433	$[\text{E2}]$	threshold of E2 to suppress GnRH release
5	$T_{P4}^{GnRH,1}$	0.0294	$[\text{P4}]$	threshold of P4 to allow E2 suppression of GnRH release
6	$m_{P4}^{GnRH,2}$	1.503	$1/[t]$	maximum part of GnRH synthesis rate constant inhibited by P4
7	$T_{P4}^{GnRH,2}$	0.0309	$[\text{P4}]$	threshold of P4 to inhibit GnRH release directly

Continued on next page...

Table B.1 – *continued from previous page*

No.	Symbol	Value	Quantity	Explanation
8	$m_{E2}^{GnRH,2}$	1.5	$\frac{[GnRH_{Pit}]}{[GnRH_{Hypo}]}$	maximum scaling of pituitary sensitivity for GnRH
9	$T_{E2}^{GnRH,2}$	1.276	[E2]	threshold of E2 to increase pituitary sensitivity for GnRH
10	$c_{GnRH,2}$	1.299	1/[t]	GnRH clearance rate constant in the pituitary
11	τ_{Inh}	1.5	[t]	delay of Inh in FSH synthesis
12	m_{Inh}^{FSH}	1	[FSH]/[t]	maximum FSH synthesis rate in the pituitary in the absence of Inh
13	T_{Inh}^{FSH}	0.06	[Inh]	threshold of Inh for inhibition of FSH synthesis
14	m_{P4}^{FSH}	2	1/[t]	maximum part of FSH release rate that is stimulated by P4
15	T_{P4}^{FSH}	0.0966	[P4]	threshold of P4 to stimulate FSH release
16	m_{E2}^{FSH}	0.3	1/[t]	maximum part of FSH release rate that is inhibited by E2
17	T_{E2}^{FSH}	2.846	[E2]	threshold of E2 to inhibit FSH release
18	m_{GnRH}^{FSH}	3	1/[t]	maximum part of FSH release rate that is stimulated by GnRH

Continued on next page...

Table B.1 – *continued from previous page*

No.	Symbol	Value	Quantity	Explanation
19	T_{GnRH}^{FSH}	0.4	[GnRH]	threshold of GnRH to stimulate FSH release
20	c_{FSH}	0.8	1/[t]	FSH clearance rate constant
21	m_{E2}^{LH}	1.5	[LH]/[t]	maximum part of LH synthesis that is stimulated by E2
22	T_{E2}^{LH}	0.1	[E2]	threshold of E2 to stimulate LH synthesis
23	m_{P4}^{LH}	4.5	[LH]/[t]	maximum part of LH synthesis that is inhibited by P4
24	T_{P4}^{LH}	0.0322	[P4]	threshold of P4 to inhibit LH synthesis
25	m_{GnRH}^{LH}	4	1/[t]	maximum part of LH release rate that is stimulated by GnRH
26	T_{GnRH}^{LH}	4	[GnRH]	threshold of GnRH to stimulate LH release
27	b_{LH}	0.05	1/[t]	basal LH release rate constant
28	c_{LH}	11	1/[t]	LH clearance rate constant
29	m_{FSH}^{Foll}	0.8	[Foll]/[t]	maximum increase of follicular function stimulated by FSH
30	T_{FSH}^{Foll}	0.8	[FSH]	threshold of FSH to stimulate follicular function

Continued on next page...

Table B.1 – *continued from previous page*

No.	Symbol	Value	Quantity	Explanation
31	T_{Foll}^{FSH}	0.3	[Foll]	threshold of follicular function to downscale FSH threshold
32	$m_{P_4}^{Foll}$	2.5	1/[t]	maximum part of follicular decay stimulated by P4
33	$T_{P_4}^{Foll}$	0.1127	[P4]	threshold of P4 to stimulate decrease of follicular function
34	m_{LH}^{Foll}	2.8	1/[t]	maximum part of follicular decay stimulated by LH
35	T_{LH}^{Foll}	0.525	[LH]	threshold of LH to stimulate decrease of follicular function
36	$\tau_{P_4,1}$	12	[t]	delay of P4 until stimulating PGF2 α increase
37	$m_{P_4}^{PGF2\alpha,1}$	0.3	[PGF2 α]/[t]	maximum growth rate of PGF2 α
38	$T_{P_4}^{PGF2\alpha,1}$	0.1672	[P4]	threshold of P4 to stimulate PGF2 α increase
39	$\tau_{P_4,2}$	17	[t]	delay of P4 until stimulating PGF2 α decrease
40	$m_{P_4}^{PGF2\alpha,2}$	11	[PGF2 α]/[t]	maximum decay rate of PGF2 α
41	$T_{P_4}^{PGF2\alpha,2}$	0.0966	[P4]	threshold of P4 to stimulate PGF2 α decrease
42	τ_{LH}	4.5	[t]	delay of LH in CL

Continued on next page...

Table B.1 – *continued from previous page*

No.	Symbol	Value	Quantity	Explanation
43	m_{LH}^{CL}	0.334	[CL]/[t]	maximum increase of CL stimulated by LH
44	T_{LH}^{CL}	1.2	[LH]	threshold of LH to stimulate CL increase
45	m_{CL}^{CL}	0.0334	[CL]/[t]	maximum increase of CL stimulated by itself
46	T_{CL}^{CL}	0.0651	[CL]	threshold of CL to stimulate self-growth
47	$m_{PGF2\alpha}^{CL}$	6.536	1/[t]	maximum decrease of CL stimulated by PGF2 α
48	$T_{PGF2\alpha}^{CL}$	2	[PGF2 α]	threshold of PGF2 α to initiate decrease of CL
49	c_{CL}^{P4}	3.856	$\frac{[P4]/[CL]}{1/[t]}$	proportionality factor of CL in P4 increase
50	c_{P4}	2.737	1/[t]	P4 clearance rate constant
51	c_{Foll}^{E2}	1.9	$\frac{[E2]/[Foll]}{1/[t]}$	proportionality factor of follicular function in E2 increase
52	c_{E2}	0.9	1/[t]	E2 clearance rate constant of
53	c_{Foll}^{Inh}	4.8	$\frac{[Inh]/[Foll]}{1/[t]}$	proportionality factor of delayed follicular function in Inh increase
54	c_{Inh}	4	1/[t]	Inh clearance rate constant

480 **Appendix C. List of equations**

481 The equations listed below are the full notations of the equations de-
 482 veloped in Section 3.2. Parameters are denoted with p and are numbered
 483 according to Table B.1. Components numbering and initial values can be
 484 found in Table C.2.

no	component	initial value
1	$GnRH_{Pit}$	1.598
2	$GnRH_{Blood}$	0.05003
3	FSH_{Pit}	0.3994
4	FSH_{Blood}	0.7996
5	LH_{Pit}	20.38
6	LH_{Blood}	0.1096
7	$Foll$	0.3988
8	$PGF2\alpha$	0.03992
9	CL	0.9808
10	P_4	0.9995
11	E_2	0.009995
12	Inh	0.1001

Table C.2: Initial values

$$\begin{aligned} \frac{d}{dt}y_1(t) &= p_2 \cdot \left(1 - \frac{y_1(t)}{p_1}\right) - \left(p_3 \cdot (h^-(y_{10}(t); p_5, 2) + h^-(y_{11}(t); p_4, 2)) \right. \\ &\quad \left. - h^-(y_{10}(t); p_5, 2) \cdot h^-(y_{11}(t); p_4, 2)) + p_6 \cdot h^-(y_{10}(t); p_7, 2)\right) \cdot y_1(t) \end{aligned}$$

$$\begin{aligned} \frac{d}{dt}y_2(t) &= \left(p_3 \cdot (h^-(y_{10}(t); p_5, 2) + h^-(y_{11}(t); p_4, 2)) \right. \\ &\quad \left. - h^-(y_{10}(t); p_5, 2) \cdot h^-(y_{11}(t); p_4, 2)) \right. \\ &\quad \left. + p_6 \cdot h^-(y_{10}(t); p_7, 2)\right) \cdot y_1(t) \cdot p_8 \cdot h^+(y_{11}(t); p_9, 5) - p_{10} \cdot y_2(t) \end{aligned}$$

$$\begin{aligned} \frac{d}{dt}y_3(t) &= p_{12} \cdot h^-(y_{12}(t - p_{11}); p_{13}, 2) - \left(p_{14} \cdot h^+(y_{10}(t); p_{15}, 2) \right. \\ &\quad \left. + p_{16} \cdot h^-(y_{11}(t); p_{17}, 2) + p_{18} \cdot h^+(y_2(t); p_{19}, 1)\right) \cdot y_3(t) \end{aligned}$$

$$\begin{aligned} \frac{d}{dt}y_4(t) &= \left(p_{14} \cdot h^+(y_{10}(t); p_{15}, 2) + p_{16} \cdot h^-(y_{11}(t); p_{17}, 2) \right. \\ &\quad \left. + p_{18} \cdot h^+(y_2(t); p_{19}, 1)\right) \cdot y_3(t) - p_{20} \cdot y_4(t) \end{aligned}$$

$$\begin{aligned} \frac{d}{dt}y_5(t) &= p_{21} \cdot h^+(y_{11}(t); p_{22}, 2) + p_{23} \cdot h^-(y_{10}(t); p_{24}, 2) \\ &\quad - (p_{27} + p_{25} \cdot h^+(y_2(t); p_{26}, 2)) \cdot y_5(t) \end{aligned}$$

$$\frac{d}{dt}y_6(t) = (p_{27} + p_{25} \cdot h^+(y_2(t); p_{26}, 2)) \cdot y_5(t) - p_{28} \cdot y_6(t)$$

$$\frac{d}{dt}y_7(t) = p_{29} \cdot h^+(y_4(t); p_{30} \cdot h^-(y_7(t); p_{31}, 1), 2)$$

$$- (p_{32} \cdot h^+(y_{10}(t); p_{33}, 2) + p_{34} \cdot h^+(y_6(t); p_{35}, 2)) \cdot y_7(t)$$

$$\frac{d}{dt}y_8(t) = p_{37} \cdot h^+(y_{10}(t - p_{36}); p_{38}, 2) - p_{40} \cdot h^+(y_{10}(t - p_{39}); p_{41}, 10) \cdot y_8(t)$$

$$\frac{d}{dt}y_9(t) = p_{43} \cdot h^+(y_6(t - p_{42}); p_{44}, 2) + p_{45} \cdot h^+(y_9(t); p_{46}, 1)$$

$$- p_{47} \cdot h^+(y_8(t); p_{48}, 2)$$

$$\frac{d}{dt}y_{10}(t) = p_{49} \cdot y_9(t) - p_{50} \cdot y_{10}(t)$$

$$\frac{d}{dt}y_{11}(t) = p_{51} \cdot y_7(t) - p_{52} \cdot y_{11}(t)$$

$$\frac{d}{dt}y_{12}(t) = p_{53} \cdot y_7(t) - p_{54} \cdot y_{12}(t)$$

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