All in good time

Dynamics of the bovine estrous cycle investigated with a mathematical model

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Dynamics of the bovine estrous cycle investigated with a mathematical model

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Abstract

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Bovine fertility is subject of extensive research in animal sciences, especially since a decline in dairy cow fertility has been observed during the last decades. One factor is reduced expression of estrous behavior. Fertility is a complex process, regulated by interactions between brain and reproductive organs. The objective of this thesis was to improve insight in the regulation of dairy cow fertility by developing and using a mechanistic mathematical model of the bovine estrous cycle. The model that was developed describes the dynamics of the bovine estrous cycle on individual cow level. It simulates follicle and CL development and the periodic changes in hormone levels that control these processes by a set of linked differential equations. The model captures a number of key physiological processes of the bovine estrous cycle, and serves as a starting point for further simulation studies, model validation, and extended models. The model was used to find candidate mechanisms that regulate follicular development. A normal estrous cycle contains 2 or 3 waves of follicular development, but why some cycles consist of 3 and others of 2 waves is unknown. Results showed that variation of (combinations of) model parameters regulating follicle growth rate or time point of CL regression can change the model output from 3 to 2 waves of follicular growth in a cycle. Several factors may perturb the regular oscillatory behavior of a normal estrous cycle. Such perturbations are likely the effect of simultaneous changes in multiple parameters. It was investigated how multiple parameter perturbation changes the behavior of the estrous cycle model, so as to identify biological mechanisms that could play a role in the development of cystic ovaries, a common reason for reproductive failure in dairy cows. Simulation results indicated that CL functioning, luteolytic signals, and GnRH synthesis are likely involved in the development of cystic ovaries. Empirical data of individual cows was used to identify mechanisms that explain individual differences in cycle characteristics by fitting the model to the data. Finding specific parameter configurations for individual cows shows the capability of the model to simulate 'real' data. Certain combinations of estimated parameter values induced a clear qualitative shift in model behavior (e.g. a different number of follicular waves), suggesting possible routes how environmental or genetic influences could affect estrous cycle characteristics. Experimental data to verify simulation results are not always available, but hypotheses based on the model predictions could be investigated in future animal experiments.

Abbreviation key

CL: corpus luteum (in the model representing the capacity of the CL to produce P4, rather than the physical size of the CL)
E2: estradiol
Foll: follicle (follicular function, in the model representing the combined capacity of all follicles present at any time to produce E2 and Inh)
FSH: follicle stimulating hormone
GnRH: gonadotropin releasing hormone
IGF-1: insulin-like growth factor-1
Inh: inhibin
IOF: interovarian factors (in the model representing several local factors, such as endothelin-1-system, cytokines, and nitric oxide, that mediate the effect of PGF2α on the CL)
LH: luteinizing hormone
OT: oxytocin
OTR: oxytocin receptor (in the model representing the overall OT-mediated mechanism in the endometrium involved in the production of PGF2α)
PGF2α: prostaglandin F2α
P4: progesterone

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General introduction

1.1 Fertility in dairy cows

Bovine fertility is the subject of extensive research in animal sciences, especially because worldwide fertility of dairy cows has declined during the last decades (for reviews see Pryce et al., 2004; Royal et al., 2000; Veerkamp et al., 2003). Subfertility of dairy cows is a significant problem for dairy farmers as it results in extra work, higher (veterinary) costs, higher risk of culling, decreased milk production and lower number of calves born. Using economic modeling of consequences of reproductive performance of dairy cows on farm profit, Inchaisi et al. (2010) showed that conception rates and estrus detection rates have a large effect on calving interval and hence on economic losses due to decreased milk production (Inchaisri et al., 2010). Poor reproductive performance thus has negative implications for dairy farm profitability and sustainability of milk production. Dairy cow fertility involves multiple factors, including resumption of ovarian activity after parturition, expression of estrous behavior to signal optimal time of insemination, and oocyte and embryo quality. Aspects that potentially influence these factors are for example genetic selection, feeding, energy balance, housing conditions, management, and metabolic and infectious diseases. Reproduction biologists, nutritionists and geneticists have put effort into gaining understanding of the underlying biological mechanisms that contribute to declined fertility in dairy cows. Although some key factors have been identified, like negative energy balance and poor detection of estrus, the complex interactions of genetic, environmental and management factors make it difficult to determine the exact physiological background for the decline in fertility (reviewed by Walsh et al., 2011).

Fertility is thus a complex process, regulated by interactions between brain and reproductive organs. Any effect interfering with one of these interactions will also affect the overall fertility outcome. The decline in fertility of dairy cows is manifested by factors like disturbed hormone patterns during the estrous cycle, reduced expression of estrous behavior and lower conception rates (Wiltbank et al., 2006). One reason why selection for higher milk yield could coincide with a decline in fertility is that high milk producing cows are more likely to have a negative energy balance during the first part of the lactation. An important cause of subfertility is metabolic stress, e.g. due to negative energy balance (Veerkamp et al., 2003), but the mechanisms by which higher milk yield can result in poorer fertility are poorly understood. Another aspect of subfertility is the low expression level of estrous behavior. Low expression of estrous behavior leads to unobserved heat and consequently suboptimal time of insemination and prolonged calving intervals. The current low estrus detection rate has been identified as an important factor decreasing reproductive efficiency (Lopez et al., 2004). Summarizing, the

reproductive physiology of cows is described extensively and there are many factors that are known to cause subfertility. However, it is not fully elucidated which physiological mechanisms regulating the estrous cycle underlie subfertility in cows.

1.2 Disturbed estrous cycle dynamics as cause of subfertility

The bovine estrous cycle is the (neuro)endocrine controlled recurrent period during which the cow prepares 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 transported through peripheral and portal blood. A normal cycle includes 2 or 3 wave-like patterns of follicle development, in which per wave a new 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 2-wave cycles than in 3-wave cycles (reviewed in Adams et al., 2008). The first 1 or 2 waves produce a dominant follicle that does not ovulate, but undergoes regression under elevated levels of P4 produced by the CL. The dominant follicle in the last wave produces increasing amounts of E2, triggering GnRH release from the hypothalamus and hence the surge of LH from the pituitary, which induces ovulation. After successful ovulation, the remains of the follicle form a new P4-producing CL (see Chapter 2 for references). Genetic variation and environmental factors cause a natural variation between cows and between estrous cycles in peak levels of hormones, number of follicular waves, etc. However, several factors may disturb the regular oscillatory nature of the bovine estrous cycle (recently reviewed by Walsh et al., 2011) and negatively affect reproductive performance. Specific changes in the dynamics of the estrous cycle, like a prolonged luteal phase, delayed ovulation, or insufficient LH release, can cause subfertility. Because the regulation of the estrous cycle is controlled by the interplay of various organs and hormones, the outcomes of specific changes in the complex physiological network underlying fertility are difficult to predict.

1.3 Mathematical modeling to understand estrous cycle dynamics

The functioning of a complex system like the bovine estrous cycle cannot be grasped in an animal experiment, although those experiments are essential to obtain quantitative data. A mathematical model can be a powerful tool for exploring the dynamic behavior of a complex biological network like the bovine estrous cycle. The integration of experimental and computational research, aiming at a better understanding of biological systems, is called a systems biology approach (Kitano, 2002). Systems biology is a relatively new research area in the field of

reproduction physiology. Systems biology approaches, including the use of mathematical models, can help to increase the understanding of the complex interplay of factors involved in the bovine estrous cycle and identify critical points in the regulation of fertility. 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, to generate hypotheses, and to identify new research questions. One advantage is that such a model describes the interactions quantitatively rather than qualitatively. Moreover, simulations with a model that integrates the components of such a complex network can reveal emerging properties of the biological system that cannot be extrapolated from the functioning of individual parts. Although the endocrine and physiologic regulation of the bovine estrous cycle is studied extensively, mathematical models of cycle regulation are scarce and of limited scope (reviewed in Chapter 3). An example of a systems biology approach in the field of reproduction physiology is found in the paper of Reinecke and Deuflhard (2007), who described a mathematical model for the human menstrual cycle. This model describes the dynamics of hormones, enzymes, receptors, and follicular phases throughout the cycle in a set of differential equations. Such a mathematical model of the bovine estrous cycle will provide new insights in the functioning of the hypothalamus-pituitary-ovarian axis, increase our understanding of the dynamics of the bovine estrous cycle, and help to design experiments.

1.4 Objective

The objective of this thesis is to improve insight in the regulation of dairy cow fertility by developing and using a mechanistic mathematical model of the bovine estrous cycle. Such a model represents a computable set of hypotheses and assumptions based on quantitative experimental data and literature. A normal estrous cycle is prerequisite for the expression of estrous behavior. Although the project was started because of interest in the regulation of estrous behavior, it was therefore chosen to focus on regulation of the estrous cycle and on causes of atypical cycles. The studies in this thesis thus focus on the causal steps of follicle and corpus luteum development and reproductive hormone releases. The mathematical model of the bovine estrous cycle that we want to develop should function as a tool to generate hypotheses, test ideas and gain novel insights that are worth further exploring. Such a model can thereby help to find causes of declined fertility in dairy cows. It can also

be used as a basis for more elaborate models with the ability to study effects of external manipulations and genetic differences.

1.5 Outline of the thesis

Reduced expression of estrous behavior is an important factor by which declined fertility is manifested and is likely affected by reproductive hormone releases (i.e., the estrous cycle). Endocrine regulation of the bovine estrous cycle is well described, but a clear understanding of how this is tied to estrous behavior is only starting to emerge. Changes in systems that regulate estrous behavior could be manifested by altered gene expression. Chapter 2 of this thesis is a literature review, describing the current knowledge on mechanisms and genes involved in the regulation of the cycle and estrous behavior. This review also describes some key factors of the physiological model that served as the starting point for the mathematical modeling process, and briefly mentions the effect of nutrition and stress on fertility.

A normal estrous cycle is prerequisite for the expression of estrous behavior. Therefore it makes sense to start the modeling process by developing a model of physiological regulation of the bovine estrous cycle and put efforts into calibration and validation of this model. In Chapter 3 we present a mechanistic mathematical model of the bovine estrous cycle that includes the processes of follicle and CL development and the key hormones that interact to control these processes.

The model described in Chapter 3 was parameterized in an iterative process using input curves based on published data for endocrine profiles of cows with a normal estrous cycle. This initial parameterization of the model simulates cycles with 3 waves of follicular development per cycle. A normal bovine estrous cycle contains 2 or 3 waves of follicle development, but the reason for cycles being of the 2 or 3 waves type is unclear. In Chapter 4 we study effects of varying key parameters on model output to investigate possible physiological mechanisms that regulate the number of waves.

Cystic ovaries is a common reason for reproductive failure in dairy cows, but the exact pathogenesis of cyst development is unclear. In Chapter 5 a multi-dimensional parameter perturbation was performed to investigate how much the parameter values could be altered before a P4 pattern associated with cystic ovaries occurred. This identified candidate biological mechanisms that could play a role in the development of cystic ovaries.

The objective of Chapter 6 was to fit the model with reasonable parameter values to data of individual animals that, although cycling normally, show

significant differences in for example peak levels and hormone profiles over time. Parameter fitting to measurements of individual animals shows that the model is able to deal with normal variation in reproductive performance in cattle and produces reasonable output parameters (for example estrous cycles with 2 or with 3 waves of follicular development). Thereby the model can help to explore biological and genetic variation with respect to fertility.

Finally, in Chapter 7 recent model developments and future perspectives are discussed. We reflect how the model can help to answer biological questions and to unravel biological mechanisms regarding fertility in dairy cows. The discussion is ended with recommendations for extending the model with energy metabolism and estrous behavior.

2

Estrous behavior in dairy cows: identification of underlying mechanisms and gene functions

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Abstract

Selection in dairy cattle for a higher milk yield has coincided with declined fertility. One of the factors is reduced expression of estrous behavior. Changes in systems that regulate the estrous behavior could be manifested by altered gene expression. This literature review describes the current knowledge on mechanisms and genes involved in the regulation of estrous behavior. The endocrinological regulation of the estrous cycle in dairy cows is well described. Estradiol is assumed to be the key regulator that synchronizes endocrine and behavioral events. Other pivotal hormones are, for example, P4, GnRH and IGF-1. Interactions between the latter and E2 may play a role in the unfavorable effects of milk yield-related metabolic stress on fertility in high milk-producing dairy cows. However, a clear understanding of how endocrine mechanisms are tied to estrous behavior in cows is only starting to emerge. Recent studies on gene expression and signaling pathways in rodents and other animals contribute to our understanding of genes and mechanisms involved in estrous behavior. Studies in rodents, for example, show that estrogen-induced gene expression in specific brain areas such as the hypothalamus play an important role. Through these estrogen-induced gene expressions, E2 alters the functioning of neuronal networks that underlie estrous behavior, by affecting dendritic connections between cells, receptor populations and neurotransmitter releases. To improve the understanding of complex biological networks, like estrus regulation, and to deal with the increasing amount of genomic information that becomes available, mathematical models can be helpful. Systems biology combines physiological and genomic data with mathematical modeling. Possible applications of systems biology approaches in the field of female fertility and estrous behavior are discussed.

Key words: dairy cow, estrous behavior, physiology, genomics

Implications

In dairy cows, optimal time of artificial insemination is signaled by estrous behavior. Selection for milk yield has coincided with a decline in duration and intensity of estrus, decreasing success of insemination. Hormonal regulation of the estrous cycle in cows is well-described, but a clear understanding of how this is tied to estrous behavior is only starting to emerge. This study reviews mechanisms and genes involved in the regulation of estrous behavior in farm animals and rodents.

2.1 Introduction

Dairy cattle selection for higher milk yield has coincided with a decline in fertility (for reviews see Royal et al., 2000; Veerkamp et al., 2003; Pryce et al., 2004). Subfertility in modern dairy cows is a multifactorial problem. It involves factors like genetic improvement for milk yield, nutritional issues, disease, season, climate, housing, management and herd environment (Lucy, 2001; Roche, 2006). The mechanisms by which selection for higher milk yield can result in poorer fertility are not totally elucidated, but one cause is likely to be metabolic stress (Veerkamp et al., 2003). As the reproductive and somatotropic axes interact at several levels in the hypothalamus (Chagas et al., 2007), it is not surprising to find relationships between energy balance and fertility parameters. 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.

Low estrus detection rate has been identified as an important factor affecting the reproductive efficiency (Lopez et al., 2004). The optimal timing of artificial insemination is signaled by estrous behavior. However, the detection of estrus in modern high milk-yield dairy cows is hampered, because the duration and intensity of estrous behavior in these cows is considerably lower than that in dairy cows of a few decades ago (reviewed by Lopez et al., 2004). Little is known about heritability and genetic variance of estrous behavior. A recent study reported heritability estimates for estrus duration and intensity to be low (2% to 8%; Lovendahl and Chagunda, 2009). Heritability estimates of fertility traits based on artificial insemination service dates are generally below 5%, while heritability estimates of days from calving to first estrous based on P4 profiles or behavior observation are higher (16% to 28%; reviewed by Pryce and Veerkamp, 2001). However, genetic control of estrous behavior as such remains elusive. Changes in the underlying mechanisms that regulate estrous behavior could be manifested by altered gene expression patterns. The study of these gene expression changes could be a means to gain insight into the genomic regulation of estrous behavior in cows. Gene expression studies are useful for discovering the biological principles

that underlie polygenic traits (Bertani et al., 2004) like estrus. There is limited information on genes that regulate the reproductive behavior in dairy cows, but considerable knowledge is available from other species, especially the rodents. The genes found to be important in rodents and other mammals may also be relevant for reproductive behavior in cows, because of shared neurophysiologic mechanisms.

The aim of this review is to describe the current state of knowledge regarding genomic regulation of estrous behavior in the brain. The first part of this study briefly summarizes the physiological mechanisms involved in estrous behavior of dairy cows, which provides the framework for the main topic: an overview of the current knowledge on relevant genes and their functions in endocrine mechanisms that regulate estrous behavior.

2.2 Physiological regulation of the estrous cycle and estrous behavior

2.2.1 General principles of estrus regulation

During pro-estrus, when the CL is regressed and the concentration of P4 is decreased, the dominant follicle, deviated from a cohort of antral follicles, matures under the influence of LH and FSH (Allrich, 1994). FSH plays an important role at the beginning of follicular development, whereas LH is important for follicular growth up to ovulation (Ginther et al., 1996). The dominant follicle secretes an increasing amounts of E2 during the development to preovulatory size (Allrich, 1994). E2 is involved in important neuroendocrine mechanisms regulating estrus. E2 inhibits GnRH secretion from the hypothalamus and LH secretion from the pituitary throughout most of the cycle. However, during pro-estrus, elevated E2 levels increase the secretion of GnRH, which together with direct effects of E2 on the pituitary, triggers the LH surge (Glidewell-Kenney et al., 2007), which induces ovulation. Once an oocyte is successfully ovulated, the remains of the follicle form a new P4-producing CL. Progesterone maintains the readiness of the endometrium for receiving the embryo. If conception has failed, the CL regresses, P4 levels decrease and the cycle restarts.

2.2.2 Estrous behavior

The estrous cycle of cows lasts for approximately 21 days. The interval between onset of mounting behavior and ovulation in cows is approximately 27 h (Lopez et al., 2002; Roelofs et al., 2005b). In modern Holstein cows the duration of estrus, defined as the time between first and last recorded standing event, has been reported to be 7 h (Dransfield et al., 1998; Lopez et al., 2004). In contrast, Esslemont and Bryant (1976) reported an average duration of estrus of 14.9 h in

Friesian cattle in 1976. Table 2.1 summarizes different behavioral signs of estrus in cows. At the start of estrus, a cow typically sniffs the vulva of other cows and rests her chin on the back of others. Such behavior is followed by mounting of other cows and ultimately the cow displays standing heat (Roelofs et al., 2005b). Van Eerdenburg et al. (1996) defined a protocol based on these behavioral signs in order to detect whether a cow is in heat. As shown in Table 2.1, not all cows express all behavior traits. Lyimo et al. (2000) and Roelofs et al. (2004) showed that the highest behavioral score of cows in estrus, based on the estrous behavior signs given in Table 2.1, correlates positively with maximum plasma E2 concentrations, but no correlation was found between E2 levels and specific estrous behaviors (Cook et al., 1986; Coe and Allrich, 1989). Because the percentage of cows displaying standing heat has declined over the last decades (reviewed by Dobson et al., 2008), it is more difficult to detect estrus based on standing heat. Therefore, other methods to detect and quantify estrus have been proposed by Roelofs et al. (2005a; pedometers) and Lovendahl and Chagunda (2009; electronic activity tags). Little is known about the underlying mechanisms and the level of genetic control of specific estrous behaviors, but collection of quantifiable data could be helpful in the research of genetic mechanisms (Schutz and Pajor, 2001).

A 'normal' endocrinological cycle is prerequisite for estrus and estrous behavior. However, ovulation is not necessarily accompanied by estrous behavior ('silent estrus'; Allrich, 1994), indicating that physiological events and behavior are in part based on different mechanisms. In dairy cows, the first postpartum ovulation occurs often without clear signs of estrous behavior (Kyle et al., 1992). This 'silent estrus' is thought to be a result of high E2 concentrations from fetal origin at the end of gestation, which induces 'refractoriness' in the hypothalamus to E2 at the first postpartum ovulation. The CL produced after the first ovulation provides the P4 that removes this refractory state and facilitates the behavioral expression of the subsequent estrus (Allrich, 1994).

Table 2.1 *behavioral signs of estrus in cows*.*

Estrous signs	Percentage of estruses in which the behavior is displayed
Flehmen	44
Sniffing vulva of another cow	100
Mounted but not standing	56
Resting with chin on back of another cow	100
(Attempt to) mount another cow	90
(Attempt to) mount head side of another cow	22
Standing heat	56

*Adapted from Van Eerdenburg *et al.*, 2002 and Roelofs *et al.*, 2005a

2.2.3 Endocrine regulation of estrous behavior: the central role of E2

E2 plays a key role in the regulation of endocrine and behavioral events associated with the estrous cycle. In many experiments that are performed to study the female reproduction, estrus is artificially induced by administering E2 (e.g. Fabre-Nys et al., 1993). E2 plays a central role in triggering the gonadotropin surge and ovulation as well as in facilitating the estrous behavior, and thus E2 indirectly synchronizes mating and ovulation. The patterns of GnRH synthesis and pulsatile release from the hypothalamus are mainly regulated by E2 (Smith and Jennes, 2001) and P4 (Richter et al., 2005; Zalanyi, 2001). E2 stimulates LH synthesis, but at levels below a certain threshold value it inhibits the release of LH. Above this threshold, the inhibitory effect on LH release switches to a stimulatory effect (reviewed by Reinecke and Deuflhard, 2007), which results in the LH surge. The shift from inhibition to stimulation may be dependent on the site of action of E2, that is, a switch from membrane signaling to genomic signaling (Arreguin-Arevalo and Nett, 2006). The LH surge is driven by an increased pituitary responsiveness to GnRH, which is determined by the amount of GnRH receptors (GnRH-Rs) expressed on gonadotropes. Pulsatile GnRH release, facilitated by high E2 concentrations during the preovulatory period, elevates GnRH-R gene expression, whereas high P4 concentrations in the luteal phase inhibits the GnRH-R gene expression (reviewed by Weiss et al., 2006).

E2 plays a pivotal role in the induction of estrous behavior (Pfaff, 2005). It has a self-amplifying effect as it stimulates the expression of estrogen receptors (ERs) in the brain, which is thoroughly investigated in rodents (Pfaff et al., 2008). The duration of estrous behavior in sheep was found to depend mostly on the duration of E2 presence rather than on its maximum concentration (Fabre-Nys et al., 1993). The effects of E2 are highly similar in different species, although threshold concentrations for the induction of estrous behavior may vary between animal species, for example, 0.4 mg/kg in sheep and 10mg/kg in rats (Fabre-Nys and Gelez, 2007). In sheep, Saïd et al. (2007) demonstrated that estrous behavior required lower E2 concentrations than required for the LH surge and that estrous behavior can be induced independently of the LH surge. Estrous behavior and the LH surge cannot only be separated by experimental reduction of E2 levels, but also by stress (Dobson et al., 2008). Lameness, an example of a stress inducing condition, was found to reduce behavior score (based on signs given in Table 2.1) of cows in estrus (Walker et al., 2008) and to inhibit LH surge and ovulation (Dobson et al., 2008) whereas incidence of estrus was not reduced (Walker et al., 2008), which could result in lower pregnancy rates. These observations suggest

that stress, caused by lameness, reduces P4 exposure before estrus (Walker et al., 2008) and/or E2 production by the dominant follicle and thereby reduces expression of estrous behavior (Dobson et al., 2008).

In cows and other domestic ruminants, the behavioral expression of estrus is preceded by a luteal phase of 12 to 15 days during which P4 concentrations are high (Fabre-Nys and Gelez, 2007). High P4 concentrations during the luteal phase inhibit the E2-induced gonadotropin surge by reducing pituitary responsiveness to GnRH (Attardi et al., 2007; Richter et al., 2005). The duration of P4 presence and the P4 amplitude in the luteal phase influence the time interval between rise in E2 levels and the induction of estrous behavior and the LH surge, probably by affecting the neural mechanisms that are involved in GnRH release (Skinner et al., 2000). The exact functions of P4 in the priming as well as the inhibition of estrous behavior are debated (Zalányi, 2001; Weiss et al., 2006; Attardi et al., 2007) and seem to differ between species (Fabre-Nys and Gelez, 2007).

2.2.4 Metabolic disturbances

High milk production affects the energy metabolism, which can disturb the endocrine signaling (Roche, 2006). Altered energy metabolism in high milk yielding cows can cause decreased levels of E2 and inhibit estrous behavior (Lopez et al., 2004). Cows selected for high milk yield are genetically induced to a more negative energy balance (Veerkamp et al., 2003) as they spend a relatively large proportion of the available nutrients on milk production, which can cause fertility problems during a period of negative energy balance (Chagas et al., 2007). One possible route by which metabolic stress can inhibit estrous behavior is via IGF-1. IGF-1 production is inhibited during negative energy balance. IGF-1 receptor signaling in the brain (Velazquez et al., 2008) is needed for the positive effect of E2 on the release of LH and for normal E2 priming of estrous behavior (Etgen et al., 2006; Mendez et al., 2006). Furthermore, concentrations of other metabolic factors that are known to affect dairy cow fertility, for example, insulin, leptin and growth hormone, interact with IGF-1 levels (Diskin et al., 2003; Chagas et al., 2007).

Changes in reproductive physiology that are associated with high milk production may in part be explained by elevated P4 and E2 clearance rates, as described in the physiological model of Wiltbank et al. (2006). In this model, clearance rates of hormones by the liver of a lactating cow are increased as a result of elevated feed intake, leading to an increased liver blood flow and metabolic activity. With a similar level of hormone production, circulating hormone levels would thus be lower. The model also provides an explanation for decreased duration of estrus: elevated E2 metabolism means a more rapid decrease in

circulating E2 after the LH surge. Combining the facts that E2 is an important regulator of estrous behavior in cows (Lyimo et al., 2000) and that increased level of milk production is associated with decreased E2 concentrations (Lopez et al., 2004), smaller follicular size (Diskin et al., 2003) and shorter duration of estrus (Wiltbank et al., 2006), it seems reasonable to conclude that lower E2 levels are (partly) responsible for the poor behavioral expression of estrus in modern dairy cows (Chagas et al., 2007).

2.3 Genomic regulation of estrous behavior: central mechanisms in the brain

General endocrinological mechanisms of the estrous cycle have been amply studied, but the understanding of the regulation of estrous behavior is only starting to emerge. Genomic approaches are often used to study physiological mechanisms. Differential expression of genes between different time points in the reproductive cycle or between animals with differences in fertility traits could indicate which genes and pathways are relevant for the regulation of estrus. This section reviews recent insights from several research areas regarding genomic regulation of estrous behavior in rodents and other mammalian species. It highlights main mechanisms, rather than dealing with all that are known to play a role, and illustrates the complex interactions between genes, hormones and their receptors that together form the signaling pathways that coordinate the synchronization of mating and ovulation.

2.3.1 Estrogen signaling in the brain

Brain areas that are known to be involved in the regulation of female sexual behavior include the arcuate nucleus (ARC), ventromedial nucleus (VMN) and preoptic area (POA) of the hypothalamus (reviewed by Molenda-Figueira et al., 2006). In addition to these areas of the hypothalamus, the hippocampus and amygdala are known to regulate the behavioral aspects of estrus. The amygdala (Zhou et al., 2005) and hippocampus (Frye and Rhodes, 2008) are involved in the reduction of anxiety and aggression, and in this way can facilitate sexual behaviors that result from generalized arousal of the brain. E2 and other hormones cause up- or down-regulation in these brain areas of a number of genes that are believed to be involved in estrous behavior (Table 2.2). E2 increases the sensitivity of neurons for itself by inducing ER gene expression (Walf and Frye, 2006 and 2008). The E2-receptor complex acts as transcription factor that regulates the expression of a large number of genes (Molenda-Figueira et al., 2006). Apart from genomic (classical ER) signaling, the estrogenic control of estrous behavior also involves membrane signaling mechanisms via secondary messengers like phosphoinositide 3

kinase, cAMP response element binding proteins and extracellular signal regulated kinases (Mendez et al., 2006; Kelly and Rønnekleiv, 2009; Micevych and Dominguez, 2009).

2.3.2 Arousal and lordosis behavior in rodents

Rodents are often used as a model to study the regulation of fertility in mammals, for example, using genomic approaches (e.g. Laissue et al., 2009). The gene expression studies of Pfaff and coworkers revealed several mechanisms that are involved in arousal (Frohlich et al., 1999) and, more specific, lordosis in rodents (Kow and Pfaff, 1998). Arousal, a general activation of brain and behavior, precedes the lordosis response and results from signaling by neurotransmitters like norepinephrine (Lee and Pfaff, 2008). The expression in the brain of estrogen receptor-α (ERα) and its downstream effects are essential for arousal, as knockout of ERα reduced arousal responses in mice (Garey et al., 2003; Mong et al., 2003a). E2-induced down-regulation of prostaglandin-D synthase in the POA increases arousal response (Mong et al., 2003b) and prostaglandin-D synthase down-regulation is associated also with lordosis (Pfaff et al., 2008). The initial step in the induction of lordosis is the E2 controlled alteration of neuronal activity in the VMN. Estrogen priming alters gene expression in VMN neurons, resulting in the activation of a variety of neurotransmitters and neuropeptides. For example, E2 induces expression of adrenergic receptor genes in the VMN (Lee et al., 2008) and increases the proportion of neurons that respond to stimulation of adrenergic receptors, which is the first step of a signal transduction pathway resulting in lordosis behavior (reviewed by Lee and Pfaff, 2008). Another example is the E2-induced expression of glial specific genes, including glutamine synthetase, in the ARC and VMN nuclei, and in the amygdala and hippocampus, thus facilitating the glutamatergic neurotransmission important for estrous behavior (Blutstein et al., 2006). At least nine genes, expressed in the rodent hypothalamus, are known to be turned on following the binding of estrogen to its receptor. In the VMN, binding of E2 to ERα activates the expression of genes for rRNA and growth, nNitric oxide synthase, adrenergic and muscarinic receptors, enkephalin and opioid receptors, P4 receptor, and oxytocin and oxytocin receptor (Table 2.2). In addition, binding of E2 to ERb activates genes for P4 receptor, and oxytocin and oxytocin receptor. In the POA, E2 binding to ERα up-regulates GnRH and GnRH-R genes and down-regulates prostaglandin-D synthase (summarized by Pfaff et al., 2008). Together, the products of these genes play a role in the induction of the behavioral expression of estrus. A recent study of Sica et al. (2009) in female mice showed changes in nNitric oxide synthase expression in the hypothalamus during the

estrous cycle. Increased numbers of nNitric oxide synthase immunoreactive neurons were found in the ARC during proestrus and in the POA during estrus. As these regions show large numbers of ERα, this study supports the conclusion of Pfaff and coworkers that E2 modulates expression of nNitric oxide synthase, which stimulates estrous behavior via activation of the nitric oxide signaling pathway (Sica et al., 2009). The estrogen-induced regulation of lordosis and synchronized ovulation in rodents can be described in five modules (Pfaff, 2005). (i) Preceding estrus, E2 induces expression of genes involved in growth of dendrites and synapses of VMN neurons that are involved in facilitating sexual behavior. (ii) P4 administration after estrogen priming amplifies the effect of estrogen on reproductive behavior via up-regulation of several transcripts. (iii) The presence of estrogens induces expression of several genes (examples are mentioned above) involved in behaviors that prepare the animal for mating. These genes establish analgesia, social recognition and reduction of anxiety and aggression. (iv) E2 induced up-regulation of neurotransmitter receptors in VMN neurons primes the neural circuit that triggers the lordosis behavior. (v) E2 elevates GnRH, which stimulates the ovulatory gonadotropin release and facilitates estrous behavior. As E2, through its effects on GnRH and LH, also regulates the LH surge and ovulation, E2 indirectly synchronizes mating and ovulation. The lordosis reflex has been used as a behavioral model to study the functioning of serotonin (Uphouse, 2000; Uphouse et al., 2007) and E2 signaling (Micevych and Dominguez, 2009; Micevych et al.,2009). Although these studies do not aim directly to unravel the regulation of estrous behavior, they support the findings of Pfaff and coworkers that E2 induces estrous behavior via ER gene expression and membrane signaling.

Near to no research has been carried out on E2-induced gene expression related to induction of estrous behavior in cattle, but there are reasons to assume that the mechanisms are similar. Estrous behavior in ruminants, like cows and sheep, is controlled by E2 levels (Lyimo et al., 2000; Saïd et al., 2007). In rodents, E2 reduces anxiety and therewith stimulates locomotion and exploratory behavior (Mong and Pfaff, 2003c), and a similar E2-induced increase in activity is seen in cows (Roelofs et al., 2005a). Another parallel that can be drawn between rodents and ruminants is that the brain areas shown to be highly involved in regulation of estrous behavior in rodents (ARC, VMN and POA) are also the regions with high concentrations of ER during estrus in ewes (Lehman et al., 1993; Stormshak and Bishop, 2008). Most gene expression studies dealing with bovine fertility are focused on follicle development and changes in ovarian tissue (Zielak et al., 2007; Mihm et al., 2006). Beerda et al. (2008) compared gene expression profiles in brain samples of Holstein Friesian heifers in (pro-)estrus to those in heifers in luteal

Table 2.2 *Overview of above-mentioned genes involved in the regulation of estrus.*

Tissue	Gene	Expression induced by	Effect	Reference
Hypothalamus	ERα, ERβ	E2	Induces expression of other genes, facilitating estrous behavior	Pfaff *et al.*, 2008
	rRNA and growth	ERα	Facilitates estrous behavior	Pfaff *et al.*, 2008
	nNitricOxide Synthase	ERα	Mediates neuro-transmission	Pfaff *et al.*, 2008; Mani *et al.*, 1994; Sica *et al.*, 2009
	Adrenergic and muscarinic receptors	ERα	Promotes neuronal excitability by modulating potassium channels	Lee and Pfaff, 2008; Pfaff *et al.*, 2008
	Enkephalin and opioid receptors	ERα	Analgesia	Pfaff, 2005; Pfaff *et al.*, 2008
	Oxytocin and its receptor	ERα, ERβ	Anxiety reduction	Pfaff, 2005, Pfaff *et al.*, 2008
	Progesterone receptor	ERα, ERβ	Stimulatory effect on lordosis	Pfaff *et al.*, 2008
	GnRH, GnRH-R	ERα	Synchronizes estrous behavior with LH peak	Pfaff *et al.*, 2008; Pfaff, 2005
	Prostaglandin-D synthase	Down-regulated by ERα	Anxiety reduction	Pfaff *et al.*, 2008, Mong *et al.*, 2003d
	Glutamine synthetase	E2	Neuro-transmission	Blutstein *et al.*, 2006
	Genes involved in PI3K pathway	E2, IGF-1	Involved in E2 signaling	Etgen and Acosta-Martinez, 2003; Malyala *et al.*, 2004
	IGF-1 receptor	E2, IGF-1	Growth of dendrites and synapses	Etgen *et al.*, 2006; Mendez *et al.*, 2006
Amygdala	Oxytocin and its receptor	ERα, ERβ	Social recognition	Pfaff, 2005
Hippocampus	Glutamine synthetase	E2	Neurotransmission	Blutstein *et al.*, 2006
Pituitary	GnRH-R	E2, GnRH	Pituitary sensitivity to GnRH	Hapgood *et al.*, 2005, Weiss *et al.*, 2006
	Progesterone receptor	E2	LH release	Attardi *et al.*, 2007

phase. Quantitative scores for estrous behavior are linked to gene expressions in the pituitary, hypothalamus, hippocampus, amygdala and ventral tegmental area (VTA). The first analyses of the VTA indicated that cyclical changes in the expression of genes regulating cell morphology and adhesion were linked to the appropriate expression of estrous behavior in dairy cows.

2.4 Understanding the complex regulation of estrous behavior

A considerable part of the reported study in this review revolves around control of ovarian E2 production and the LH surge rather than regulation of estrous behavior, because relatively little research has been carried out on the control of estrous behavior as such. Gene expression profiling is a powerful tool for the identification of genes and mechanisms underlying estrous behavior in dairy cows. The sequencing of the genome of diverse animal species provided a huge amount of data and the biological interpretation of these data has just begun. Large datasets are being generated by functional genomics approaches, like measuring differential expression of genes related to fertility. Various bioinformatics and other post-analysis approaches are being used in order to integrate these data in physiological concepts. Reproductive behavior is a result of numerous gene products, cooperating in pathways that finally induce or facilitate a behavioral response. The number of factors involved in the regulation of estrus is overwhelming and mirrors complex networks. To improve the understanding of bovine reproductive behavior, it might be supportive to integrate the involved physiological and genomic components to describe the various mechanisms that are involved in the interplay of relevant brain areas like the hypothalamus, and the pituitary and ovaries.

To understand complex biological networks like the regulation of estrus, mathematical models and simulation studies can be helpful (Potter and Tobin, 2007). Mathematical models have been developed, for example, for follicle development (Clément et al., 2001; Soboleva et al., 2004), gonadotropin release (Blum et al., 2000; Heinze et al., 1998; Washington et al., 2004), and estrogen signaling (Vasudevan and Pfaff, 2008; Frohlich et al., 2002). The coupling of physiological and genomic data with the help of modeling (e.g. gene network models or mechanistic mathematical models) aims to improve insight in the biological system as a whole (Burbeck and Jordan, 2006), and this approach is often referred to as systems biology. An interesting example of a systems biology approach in the field of female reproduction is the model for the human menstrual cycle developed by Reinecke and Deuflhard (2007), which integrates the major tissues and hormones involved, and is able to simulate the dynamics of follicular development and the associated cyclic hormone level changes. It is expected that a

systems biology approach improves the understanding of physiological consequences of alterations in gene expression patterns, for example, the possible implications for expression of estrous behavior. Systems biology approaches, including the use of network models and mechanistic mathematical models, are likely to play a role in further increasing our understanding of the complex interplay of factors involved in the reproductive cycle and the regulation of estrous behavior.

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3

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 CL development and the key hormones that interact to control these processes. The model generates successive estrous cycles of 21 days, with 3 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 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 the 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.

Key words: cow, reproduction, hormone patterns, differential equations, systems biology

3.1 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 Pryce et al., 2004; Veerkamp et al., 2003). This decline in fertility is shown by, e.g. alterations in hormone patterns during the estrous cycle, reduced expression of estrous behavior and lower conception rates (Wiltbank et al., 2006). However, it is hard to understand which underlying mechanisms cause this decline in fertility. The regulation of estrus is controlled by the interplay of various organs and hormones. Mathematical modeling of the involved mechanisms is expected to improve insight in the biological processes underlying the bovine estrous cycle, and could thereby help to find causes of declined fertility in dairy cows (Boer et al., 2010b).

Although the endocrine and physiologic regulation of the bovine estrous cycle is studied extensively, mathematical models of cycle regulation are scarce and of limited scope (Meier et al., 2009; Soboleva et al., 2000). A number of models have been developed for other ruminant species, especially ewes (Clément et al., 2002; Heinze et al., 1998), but these models do not contain all the key players that are required to simulate follicle development and the accompanying hormone levels throughout consecutive cycles. A model that integrates the major tissues and hormones involved, and that is able to simulate the dynamics of follicular development, has been developed for the human menstrual cycle by Reinecke and Deuflhard (2007). This model, which is based on previous work by Selgrade and colleagues (Selgrade and Schlosser, 1999; Schlosser and Selgrade, 2000; Clark et al., 2002), describes the dynamics of hormones, enzymes, receptors, and follicular phases throughout the cycle in a set of differential equations.

The objective of the work described in this paper was to develop a mathematical model of the dynamics of the bovine estrous cycle on individual cow level, that is able to simulate follicle development and the accompanying fluctuations in hormone concentrations. Physiologic and endocrine mechanisms

that regulate the cycle are very similar between human and cows. Therefore, some mechanisms of the human model in Reinecke and Deuflhard (2007) could be used (although sometimes with simplifications), and extended with other mechanisms like follicular wave emergence and corpus luteum regression.

Focus in this paper is on the model development. Section 3.2 describes the biological mechanisms of the bovine estrous cycle and how these mechanisms are incorporated in the model. In Section 3.3, the mathematical description and all model equations and parameters are given. Simulation results are presented in Section 3.4, showing that a set of equations and parameters is obtained that describes the system consistent with biological data for cows. In Section 3.5, it is discussed how the current model could be applied and extended.

3.2 Biological background

3.2.1 Follicles

Two different patterns of follicle development are identified in mammals. In humans (and rats and pigs), the development of follicles to ovulatory size occurs only during the follicular phase, while in cattle (and sheep and horses), the development of follicles to ovulatory or near-ovulatory size occurs throughout the cycle (Fortune, 1994). A normal cycle includes 2 or 3 wave-like patterns of follicle development, in which a cohort of follicles start to grow. The average duration of the bovine estrous cycle is 20 days for 2-wave and 22 days for 3-wave cycles (reviewed in Adams et al., 2008). Each follicular wave is initiated by an increase of FSH release from the anterior pituitary (Ginther et al., 2002). The growing follicles produce E2 and Inh, which are released into peripheral blood. In the first 1 or 2 waves, a dominant follicle deviates from the cohort of growing follicles that does not ovulate, but undergoes regression under influence of P4 produced by the CL. When the CL is regressed under influence of PGF2α, the concentration of P4 decreases (Niswender et al., 2000). The dominant follicle present at that moment develops and matures, and ovulation can then take place because the inhibiting effect of P4 on the surge of LH is removed (Mann and Lamming, 1995). Elevated E2 levels increase the secretion of GnRH, which triggers the LH surge and thereby induces ovulation. Once an oocyte is successfully ovulated, the remains of the follicle form a new P4-producing CL. If conception has failed, the CL regresses, P4 levels decrease, and the cycle restarts (reviewed in Boer et al., 2010b).

The ovaries contain a pool of small follicles with immature oocytes. Under influence of FSH, a cohort of 8-41 growing follicles emerge (Adams et al., 2008). Approximately 2 days after cohort recruitment, one follicle is selected to become the dominant follicle, and continues to grow (Bao and Garverick, 1998). This

deviation of the dominant follicle is associated with increased FSH and LH receptor binding, activating the enzymes that catalyse steroidogenesis, resulting in increased E2 production and higher E2 serum levels (Bao and Garverick, 1998). The dominant follicle expresses more FSH receptors, and it can therefore continue to grow even when FSH serum levels are low (Beg et al., 2002). In the model, the emergence of a follicular wave is induced when FSH exceeds a threshold which becomes lower when follicles become larger, representing that larger follicles are more sensitive to FSH. Dominant follicles also secrete increasing amounts of Inh. Inh suppresses FSH and, hence, suppresses the growth of subordinate follicles. Ovulation or regression of the dominant follicle eliminates this suppression, allowing the onset of the next follicular wave (Bleach et al., 2001; Ginther et al., 2001).

Small follicles of an emerging cohort may release very small amounts of E2 and Inh per follicle, but taken together, this amount is not negligible. Furthermore, there is always a medium-size or large follicle present (Ireland et al., 2000; Wise, 1987; Wolfenson et al., 2004), which results in a basal hormone production throughout the cycle. Different follicles are recruited, growing, and regressing in each cycle and in each wave. However, total E2 and Inh production capacities are modeled as a continuous function throughout subsequent waves and cycles, representing the total amount of hormone production of the follicles present at any moment. Follicle regression is promoted by high P4 levels and by the LH surge (Eq. (3.7)). The capacity of follicles to produce E2 and Inh is denoted as ‘‘follicular function’’ in the rest of this paper.

3.2.2 Corpus luteum

The CL develops within 2–3 days after ovulation, starting the synthesis and release of P4, which maintains the readiness of the endometrium for receiving the embryo. In the absence of a conceptus, the CL will regress at days 17–18 of the cycle (Miyamoto et al., 2009; Taylor and Rajamahendran, 1991). In each cycle a new CL develops, but CL development is modeled as a continuous function of P4 producing tissue, denoted as ‘‘CL function’’ in the rest of this paper. In the model, CL development is induced by the LH surge. A threshold and delay are incorporated in the effect of LH on the CL, to account for the time required for the process of transition from follicle to CL (Niswender et al., 2000) and the shift from E2 to P4 production (Dieleman et al., 1986; Dieleman and Blankenstein, 1985). If the CL reaches a certain size, it continues to grow without further stimulation by LH (Skarzynski et al., 2001). CL regression is induced by PGF2α secretion from the uterus (described in Section 3.2.4). Growth and regression of CL function are described by Eq. (3.9).

3.2.3 Estradiol and inhibin

E2 affects LH synthesis and release (Glidewell-Kenney et al., 2007) and FSH release (Beg et al., 2002; Lane et al., 2005). E2 serum levels are higher in ovulatory than in non-ovulatory waves (Bleach et al., 2001; Echternkamp and Hansel, 1973) and reach peak levels around estrus (Bleach et al., 2001; Echternkamp and Hansel, 1973; Evans et al., 1997; Glencross et al., 1981; Kanchev et al., 1976; Wise, 1987). This suggests that the preovulatory follicle has the largest capacity to produce and release E2, although its maximum size is not significantly different from the maximum size of non-ovulatory dominant follicles. Considering the results in Acosta (2007) and Acosta et al. (2000), where a better vascularity of the ovulatory follicle is reported, it is reasonable that the ovulatory follicle can secrete more E2 than non-ovulatory follicles and, consequently, E2 serum levels are the highest at estrus. In the model, the rate of E2 production and release to the blood is taken as proportional to follicular function (Eq. (3.11)).

Inh inhibits FSH synthesis and thus reduces FSH release (Ginther et al., 2001). Compared to basal Inh serum levels, peak levels are almost doubled in non-ovulatory waves and increase further in ovulatory waves (Parker et al., 2003). There are different forms of inhibin, but only inhibin A is considered in the model, as it is the predominant form in bovine follicular fluid (Beg et al., 2002). In the model, Inh production rate is taken as proportional to follicular function (Eq. (3.12)).

3.2.4 Progesterone and prostaglandin F2α

The CL is the main source of P4. Serum P4 concentration is near to zero around estrus and high during the luteal phase (Adams et al., 1992; Díaz et al., 1986; Echternkamp and Hansel, 1973; Kaneko et al., 1995; Stabenfeldt et al., 1969). A high correlation between CL diameter and P4 output was reported in Perry et al. (1991), Savio et al. (1988) and Wolfenson et al. (2004). In the model, the rate of P4 release into the blood is taken as proportional to CL function (Eq. (3.10)).

Pulsatile PGF2α release from the uterus induces CL regression. The rise of P4 early in the cycle initiates a series of events or mechanisms that eventually lead to the rise of PGF2α, followed by a decline of PGF2α a few days later. It was shown that administration of P4 prior to its natural rise resulted in an equally earlier onset of CL regression (Mann et al., 1998). Exposure to effective amounts of P4 must last for 10–13 days to induce PGF2α pulses (Mann et al., 1998; Poyser, 1995; McCracken et al., 1999; Goff, 2004). Peak PGF2α levels are 3–4 times higher than basal levels (dos Santos et al., 2009; Mann and Lamming, 2001; Shaham-Albalancy et al., 2001; Araujo et al., 2009).

PGF2α is regulated by OT, P4 and E2 (Silvia et al., 1991). P4 first prevents a too early release of PGF2α pulses, but simultaneously stimulates synthesis of enzymes required for PGF2α production. In the later luteal phase, changed expression of P4 and OT receptors results in a gradual decrease in the suppression of PGF2α (dos Santos et al., 2009), leading to an OT induced pulsatile release of PGF2α (Araujo et al., 2009; Poyser, 1995). How these mechanisms are regulating each other is quite complex and not understood in full detail.

What is clear is that the rise in P4 levels and the continued presence of P4 above an effective level sets in motion a series of events that lead to CL regression. Hence, we incorporated these series of events as a black box using time delays to obtain the right timing of PGF2α signaling. In the model, PGF2α increases a specific number of days (delay $\tau_{P4,1}$) after P4 levels reach a threshold. Similarly, PGF2α declines another (larger) number of days (delay $\tau_{P4,2}$) after P4 levels reached a threshold (Eq. (3.8)).

3.2.5 Gonadotropin releasing hormone, luteinizing hormone and follicle stimulating hormone

Pulsatile signaling of GnRH regulates LH and FSH secretion (Pawson and McNeilly, 2005). Because GnRH induces the LH surge, it indirectly induces ovulation (Troxel and Kesler, 1984). The GnRH pulse generator is located in the hypothalamus and is modulated by P4 and E2 (Goodman, 1988). During the luteal phase, both P4 and E2 suppress the activity of the GnRH pulse generator. During pro-estrus, however, elevated E2 levels change estrogen receptor signaling, which induces a GnRH surge (Glidewell-Kenney et al., 2007; Goodman, 1988). GnRH is released into the portal circulation of the pituitary and binds to GnRH receptors of the anterior pituitary (Vizcarra et al., 1977). In the model, GnRH stimulates LH release, resulting in an LH surge concurrently with the GnRH surge. GnRH synthesis is taken constant as long as the amount of GnRH in the hypothalamus is below a threshold (Eq. (3.1)). GnRH release is inhibited when P4 levels are above a threshold and when both P4 and E2 levels are above a threshold. GnRH release is stimulated when P4 levels are low and E2 reaches a threshold (Eq. (3.1b)), resulting in a surge of GnRH. GnRH concentration in the pituitary depends on GnRH amount released from the hypothalamus, and is further increased by high E2 levels, representing that E2 up-regulates expression of GnRH receptors (Goodman, 1988; Vizcarra et al., 1977) (Eq. (3.2)).

The LH surge at the day before ovulation induces ovulation of the ovulatory follicle and formation of the CL. The LH surge will shut down E2 and Inh production capacity of the ovulatory follicle (Chenault et al., 1975; Wolfenson et

al., 2004). High P4 levels suppress the release of LH via the inhibition of the GnRH pulse generator (Bergfeld et al., 1996). Additionally, high P4 levels decrease pituitary sensitivity to E2, thereby increasing the amount of E2 required to induce an LH surge above physiological levels (Goodman, 1988). Peak LH levels are about five times as high as basal levels or higher (Bleach et al., 2001; Echternkamp and Hansel, 1973; Kotwica and Williams, 1982; Dieleman et al., 1986). In the model, LH synthesis is stimulated by E2 and inhibited by P4 (Eq. (3.5a)). Besides a small basal LH release, there is a surge of LH when GnRH in the pituitary reaches a threshold (Eq. (3.5b)).

FSH synthesis is inhibited by Inh (Beg et al., 2002). P4 and E2 modulate FSH release via effects on the anterior pituitary and on the GnRH pulse generator in the hypothalamus. Peak FSH serum levels are about 3 times higher than basal levels (Bleach et al., 2001; Evans et al., 1997). In the model, FSH synthesis in the pituitary is increased when Inh levels are below a threshold (Eq. (3.3a)). FSH release from the pituitary to the blood is stimulated by P4 and GnRH, and inhibited by E2 (Eq. (3.3b)).

3.3 Mathematical formulation

The mathematical approach used for the bovine model is comparable to the approach used for the model of the human menstrual cycle, which originally has been developed at North Carolina State University by Selgrade and colleagues (Selgrade and Schlosser, 1999; Schlosser and Selgrade, 2000; Harris, 2001; Clark et al., 2002), and has been extended at the Zuse Institute (Reinecke and Deuflhard, 2007; Reinecke, 2008).

The system is considered in four compartments: hypothalamus, anterior pituitary, ovaries and uterus, connected through peripheral and portal blood (Fig. 3.1). The model includes the processes of follicle and CL development and the key hormones that interact to control these processes as described in Section 3.2. The gonadotropin equations are based on synthesis-release-clearance relations. This structure was first introduced in Schlosser and Selgrade (2000). The complete mechanisms are shown in Fig. 3.2.

Based on these mechanisms, 12 ordinary differential equations (ODEs) with 54 parameters are formulated. If necessary, time delays are incorporated to model the time between events and their effects, representing the duration of intermediate steps in biological processes. In this case, the ODE is turned into a

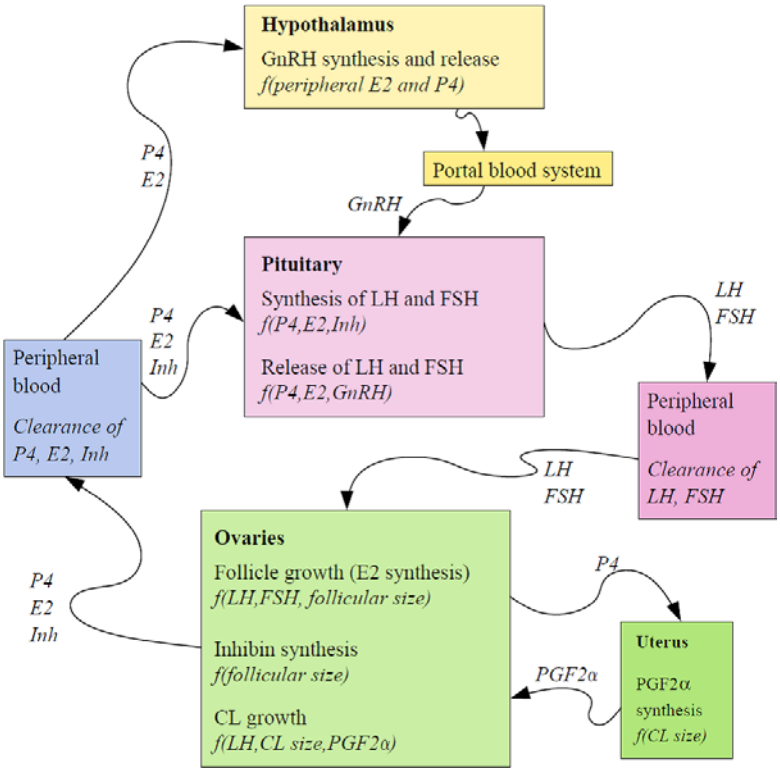

Figure 3.1 *Schematic representation of the compartments in the model of the bovine estrous cycle.*

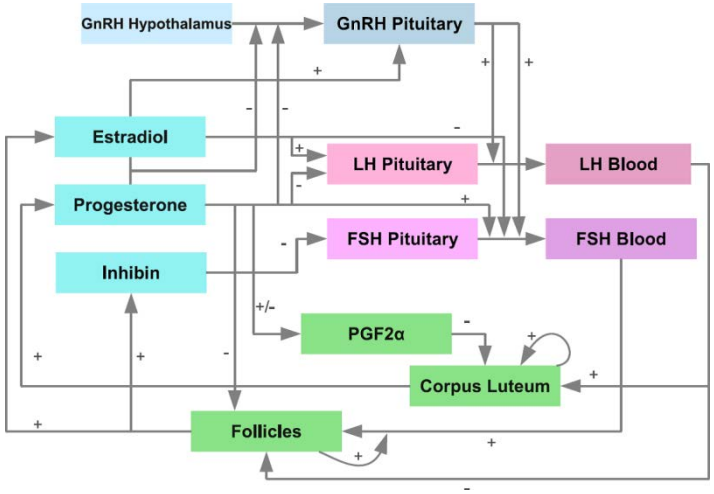

Figure 3.2 *Complete mechanisms of the bovine model. Boxes represent the 12 key components of the system. Differential equations are derived for these 12 components. Arrows denote functional dependencies. Stimulating and inhibiting effects are indicated by + and -, respectively.*

delay differential equation (DDE). To solve the system of differential equations, we use the solver RADAR5 (Guglielmi and Hairer, 2005), which has been designed for the solution of stiff delay differential equations.

3.3.1 Hill functions

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

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

S(t) represents the effector, *T* the threshold for change of behavior, and *n* controls the steepness of the curve (Fig. 3.3). Negative Hill functions are used for inhibitory effects and are defined as

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

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

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

3.3.2 Model equations

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

$$\frac{d}{dt} GnRH_{Hypo}(t) = Syn_{GnRH}(t) - Rel_{GnRH}(t) \tag{3.1}$$

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

$$Syn_{GnRH}(t) = c_{GnRH,1} \cdot \left(1 - \frac{GnRH_{Hypo}(t)}{GnRH_{Hypo}^{max}}\right) \tag{3.1a}$$

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

$$Rel_{GnRH}(t) = (H_1^-(P4\&E2) + H_2^-(P4)) \cdot GnRH_{Hypo}(t) \qquad (3.1b)$$

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

$$\frac{d}{dt} GnRH_{Pit}(t) = Rel_{GnRH}(t) \cdot H_3^+(E2) - c_{GnRH,2} \cdot GnRH_{Pit}(t) \qquad (3.2)$$

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

$$\frac{d}{dt} FSH_{Pit}(t) = Syn_{FSH}(t) - Rel_{FSH}(t) \qquad (3.3)$$

FSH synthesis rate in the pituitary is only dependent on delayed Inh, as in Harris (2001). FSH is synthesized when the Inh level is low, i.e. high Inh levels inhibit FSH synthesis, which is included as a negative Hill function,

$$Syn_{FSH} = H_4^-(Inh_\tau) \qquad (3.3a)$$

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

$$Rel_{FSH} = \left(H_5^+(P4) + H_6^-(E2) + H_7^+(GnRH_{Pit})\right) \cdot FSH_{Pit}(t) \qquad (3.3b)$$

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

$$\frac{d}{dt} FSH_{Blood}(t) = Rel_{FSH}(t) - c_{FSH} \cdot FSH_{Blood}(t) \qquad (3.4)$$

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

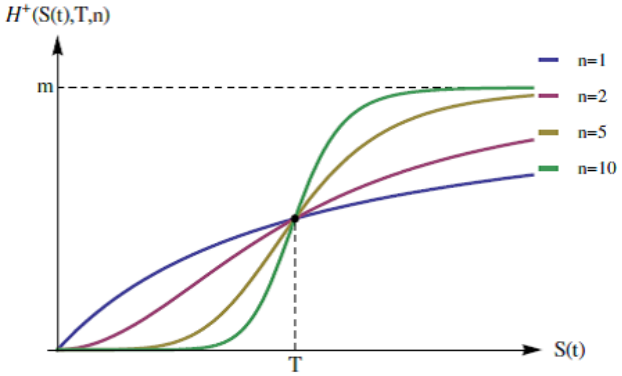

Figure 3.3 *Scaled positive Hill functions with different steepness.*

Like FSH, the LH serum level depends on synthesis in the pituitary, release into the blood and clearance thereof,

$$\frac{d}{dt}LH_{Pit}(t) = Syn_{LH}(t) - Rel_{LH}(t) \tag{3.5}$$

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

$$Syn_{LH}(t) = H_8^+(E2) + H_9^-(P4) \tag{3.5a}$$

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

$$Rel_{LH}(t) = \left(b_{LH} + H_{10}^+(GnRH_{Pit})\right) \cdot LH_{Pit}(t) \tag{3.5}$$

Summarizing, LH in the blood is obtained as

$$\frac{d}{dt}LH_{Blood}(t) = Rel_{LH}(t) - c_{LH} \cdot LH_{Blood}(t) \tag{3.6}$$

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

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

$$\frac{d}{dt}Foll(t) = H_{11}^+(FSH) - (H_{12}^+(P4) + H_{13}^+(LH_{Blood})) \cdot Foll(t) \tag{3.7}$$

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,

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

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

$$H_{11}^{+}(FSH) := m_{FSH}^{Foll} \cdot h^{+}(FSH_{Blood}(t); \tilde{T}_{FSH}^{Foll}(t), 2) \tag{3.7a}$$

PGF2α initiates the functional regression of the CL, and thereby the decrease in P4 levels. After a large time delay, PGF2α synthesis is stimulated by elevated P4 levels above a specified threshold value. The PGF2α level declines a couple of days after its rise, which is included as a delayed positive effect of P4 on the decay of PGF2α,

$$\frac{d}{dt} PGF2\alpha(t) = H_{14}^{+}(P4_{\tau 1}) - (H_{15}^{+}(P4_{\tau 2}) \cdot PGF2\alpha(t) \tag{3.8}$$

The LH peak initiates growth of the CL with a specified delay. After reaching a certain size, the CL continues to grow on its own as long as PGF2α is low. The CL starts to regress when PGF2α levels rise above a threshold,

$$\frac{d}{dt} CL(t) = H_{16}^{+}(LH_{\tau}) + H_{17}^{+}(CL) - H_{18}^{+}(PGF2\alpha) \cdot CL(t) \tag{3.9}$$

The production of P4 in the ovary is assumed to be proportional to CL function, and the production of E2 and Inh is assumed to be proportional to follicular function. Therefore, the equations for P4, E2, and Inh do not contain any Hill functions,

$$\frac{d}{dt} P4(t) = c_{CL}^{P4} \cdot CL(t) - c_{P4} \cdot P4(t) \tag{3.10}$$

$$\frac{d}{dt} E2(t) = c_{Foll}^{E2} \cdot Foll(t) - c_{E2} \cdot E2(t) \tag{3.11}$$

$$\frac{d}{dt} Inh(t) = c_{Foll}^{Inh} \cdot Foll(t) - c_{Inh} \cdot Inh(t) \tag{3.12}$$

The parameters c_{P4}, c_{E2} and c_{Inh} denote the respective clearance rate constants.

Fig. 2 gives an overview of all mechanisms described by the model equations. Detailed notations for the Hill functions, equations and parameters are given in Appendices 1A-D, respectively.

3.3.3 Parameter identification and sensitivity analysis

The main difficulty is not to simulate the system, i.e. to solve the differential equations, but to identify the unknown parameters. Unfortunately, many of the parameters are not measurable. Sometimes the range of values is known, but some parameters are completely unknown. The techniques for parameter estimation that are used in this model are implemented in the software packages PARKIN (Nowak and Deuflhard, 1985; Deuflhard and Nowak, 1986) and NLSCON (Nowak and Weimann, 1993–2004), which have been developed at the Zuse Institute for many years. These programs take into account parameter sensitivities and linear dependencies, and include a number of optimization methods such as, for example, affine covariant Gauss–Newton methods (Deuflhard, 2004). A renewed version of this software, especially adapted to parameter identification in ordinary differential equation models, has been used throughout the paper. The mathematical background is described in Deuflhard (2004).

To obtain a good initial guess for the parameter optimization procedure, we use a model decomposition approach and successively enlarge the set of estimated parameters. The first step is to define input curves representing the development of Inh, P4, and E2 levels in the blood over time. This use of explicit functions, which simplifies parameter identification, was already suggested by Schlosser and Selgrade (2000). Composition of these input curves is based on published data for endocrine profiles of cows with a normal estrous cycle, see for example Perry (2004).

Following the approach in Harris (2001), we use the input curves to successively fit the profiles of the other components. The detailed procedure can be found in Boer et al. (2010a). In the last step, the input curves for P4, E2, and Inh are replaced by their original ODE/DDE description to obtain a closed network. The final parameter values are listed in Appendix 1D, and the corresponding simulation results are illustrated in Fig. 3.4.

A sensitivity analysis has been performed with the techniques described in Deuflhard (2004). A more detailed description including column norms of the sensitivity matrix and subconditions, which provide information about the sensitivities and the dependencies of the parameters, can be found in Boer et al. (2010a). It turns out that among the most sensitive and best predictable parameters are $p_{36} = \tau_{P4,1}$, $p_{11} = \tau_{Inh}$, $p_{20} = c_{FSH}$ and $p_{39} = \tau_{P4,2}$.

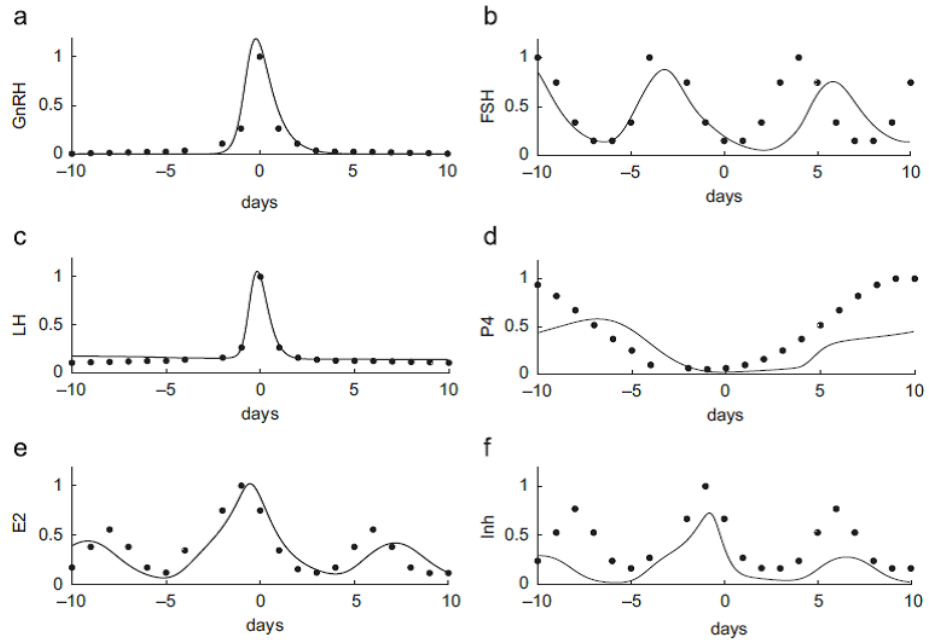

Figure 3.4 *Simulated curves of the closed model together with the data points used for parameter estimation. Panels a-c show data points based on qualitative behavior of hormones as described in the literature (for example Perry, 2004). Panels d-f show data points obtained from the input curves. Day zero corresponds to the day of LH peak.*

3.4 Simulation results

The figures in this section show the computed dynamics of follicle and CL development and accompanying fluctuations in hormone levels over consecutive cycles. The simulation results show that the current set of model parameters generates curves consistent with empirical knowledge for cows with a normal estrous cycle with 3 follicular waves. Notice that the model generates consecutive cycles that are not entirely identical (quasi-periodic behavior), but that vary slightly in patterns and peak heights between cycles. Small differences in model output at the end of a cycle result in a different starting point of the next cycle, which leads to variation between the curves. This variation in hormone levels between cycles could well resemble variation within a cow over consecutive cycles. However, a different parameterization can be used to produce a stable limit cycle.

Each estrous cycle contains 3 waves of follicular growth (Fig. 3.5). The CL starts to grow a few days after ovulation and is large during the first 2 follicular waves, which suppresses follicle growth. As the larger follicles become more sensitive to P4, at a certain size the effect of P4 becomes so large that it induces

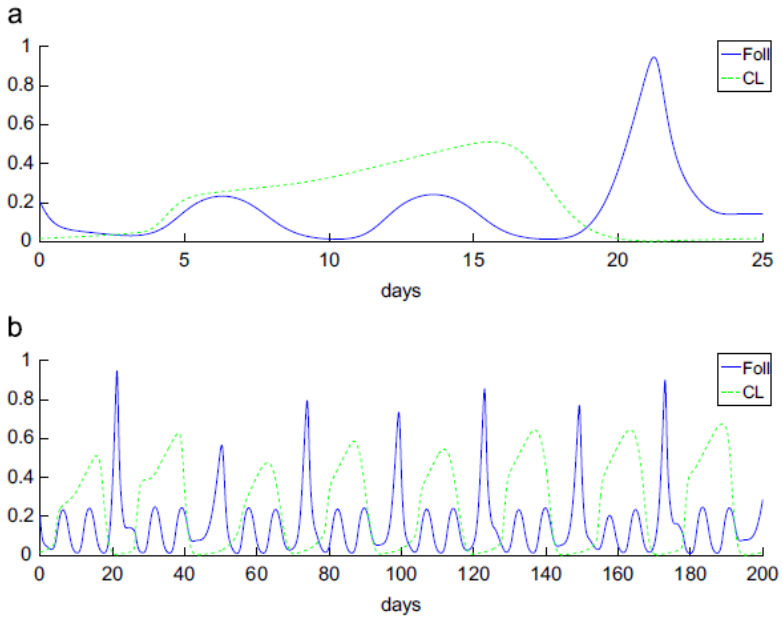

Figure 3.5 *Output curves of follicular function (Foll) and CL function (CL) over time for one cycle (a) and in consecutive cycles(b).*

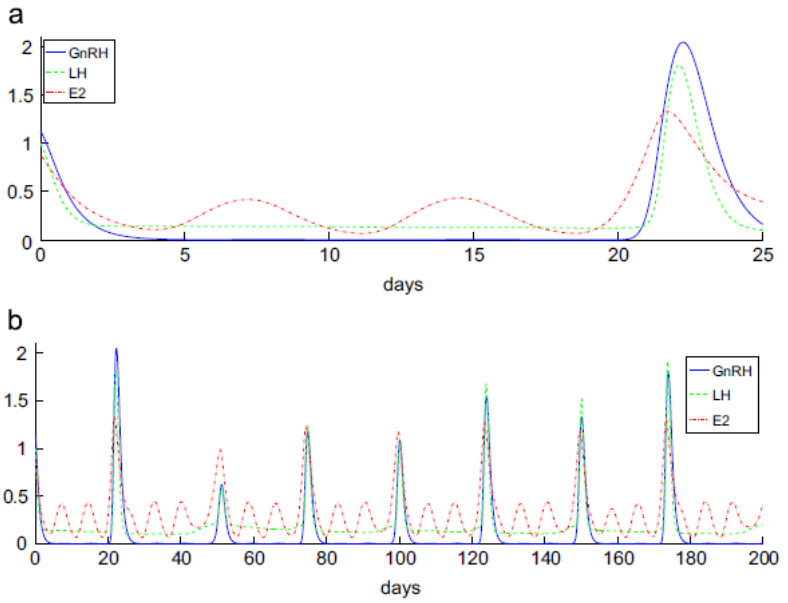

Figure 3.6 *Output curves of serum concentrations of E2 and LH, and portal concentration of GnRH over time for one cycle (a) and in consecutive cycles (b).*

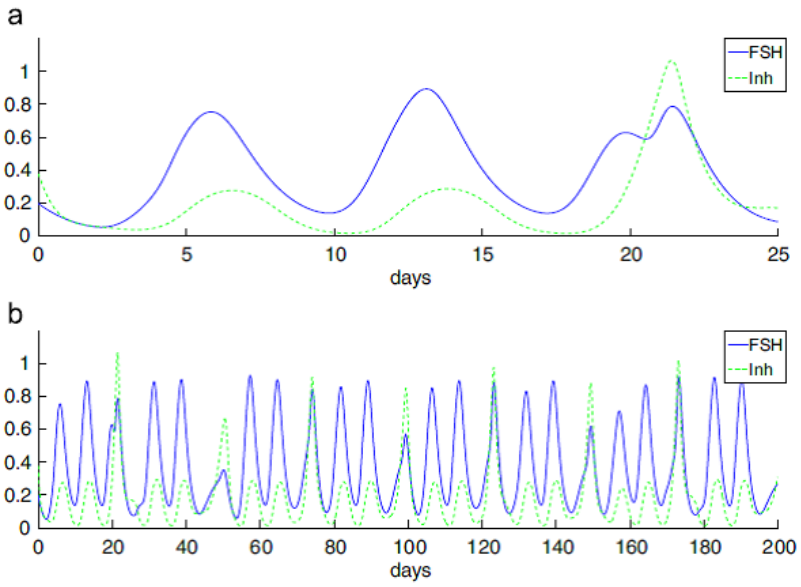

Figure 3.7 *Output curves of serum concentrations of Inh and FSH over time for one cycle (a) and in consecutive cycles (b).*

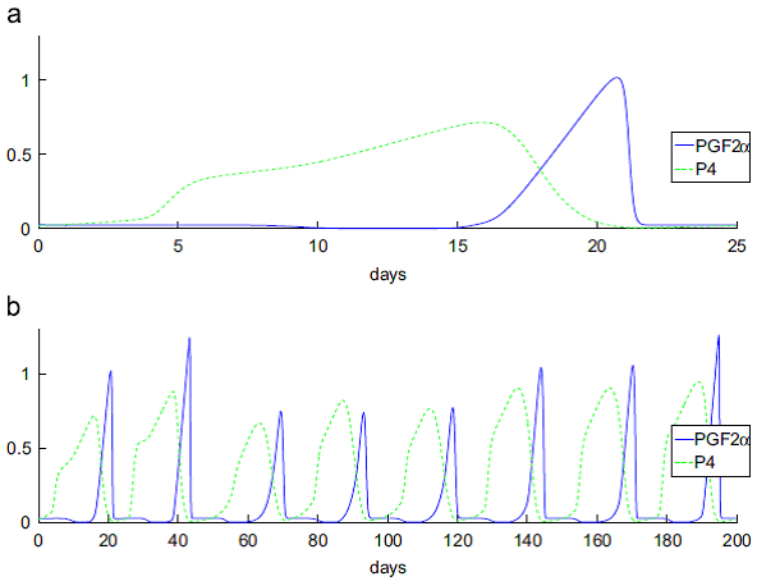

Figure 3.8 *Output curves of serum concentrations of P4 and PGF2α over time for one cycle (a) and in consecutive cycles (b).*

follicle regression. After regression of the CL, the dominant follicle of the third follicular wave can continue to grow, leading to ovulation, which causes a sharp decline in follicular function.

The pattern of serum E2 levels is a result of follicular function (Figs. 3.5 and 3.6). The third wave of follicular growth takes place when P4 levels are low, resulting in increased E2 levels. These increased E2 levels induce a steep GnRH and LH surge, which is the trigger for ovulation. Notice that the height of the GnRH surge is determined by the E2 peak level. During the remaining cycle, GnRH and LH levels are low, representing the lower pulse frequency and amplitude compared to the surge.

Increased FSH levels induces the growth of a follicular wave and thereby the start of Inh increase, but FSH is suppressed when Inh levels are above a certain level (Fig. 3.7). Notice that FSH peak levels in the third wave of the cycle differ in consecutive cycles because of corresponding differences in height of the GnRH surge (Figs. 3.5 and 3.6). When Inh has declined due to follicular regression, FSH increases again and induces the next follicular wave. Because follicular growth is modeled in 3 waves, also Inh levels rise in 3 waves in a cycle.

P4 serum levels are proportional to CL function. P4 concentration is small during the first days of the cycle and rises when the CL starts to grow (Fig. 3.5). Notice that a lower LH peak height results in a less steep P4 increase and lower levels of P4 in the following cycle (Figs. 3.6 and 3.8). Increased P4 levels induces a rise in PGF2α after a couple of days, which causes CL regression and declining P4.

3.5. Discussion and outlook

The current mathematical model describes the interaction between a number of key physiological processes of the bovine estrous cycle. The model is able to simulate the dynamics of follicle and CL growth and development, as well as the associated hormone level changes in consecutive cycles. The current model comprises 12 equations and 54 parameters. The estrous cycles generated by the model are not entirely identical and could well resemble variations within a cow over consecutive cycles.

The above simulations show a quasi-periodic behavior, but a different parameterization (not listed in this paper) could be used to produce a stable limit cycle. This shows that the variations between simulated cycles are not an intrinsic characteristic of the model, but depend on the parameterization. However, the cycles of a real cow are usually quite irregular, and we think this is not due to changes in external factors for that cow but rather 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 mimics. Alternatively, one could add a stochastic component to the regular system (representing small variations in external factors) to induce variations in consecutive cycles, but this was not in the scope of our work.

The sensitivity analysis shows that parameters 36, 20 and 11 are the most sensitive parameters of the model, which means that a small change in the value of one of these parameters will have a large effect on the model solution. Parameter 36 (delay of P4 until stimulating PGF2α increase) is possibly a sensitive parameter because CL life span is critical for the duration of the cycle. Parameter 20 (FSH clearance rate constant) and Parameter 11 (delay of Inh in FSH synthesis) are possibly sensitive parameters because FSH and Inh serum levels have an important effect on the progress of follicle development.

The modeling method with ODEs/DDEs as used for the presented model of the bovine estrous cycle was also used for the model of the human menstrual cycle (Selgrade and Schlosser, 1999; Schlosser and Selgrade, 2000; Harris, 2001; Reinecke and Deuflhard, 2007). As we aimed at the development of a model for the dynamical changes of a biological system, including the information about how components influence the rates of change of other components, our approach to model the system with differential equations appears to be the most reasonable. Maybe qualitative results could have been obtained with other methods such as, for example, boolean networks, but differential equations allow for a simulation of quantitative profiles of the involved components. To our knowledge, no comparable models of the bovine estrous cycle are available.

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

Although the current model could thus offer possibilities to simulate fertility disorders, its predictive ability may be limited in those parts and for those aspects in which the model is not entirely mechanistic but rather descriptive. One example thereof is the modeling of PGF2α. Because the detailed biological mechanisms that induce the rise of PGF2α are very complex and not completely understood, we chose to restrict the number of state variables for this part of the model, and to include time delays. This mimics the situation in cows that the rise of P4 early in the cycle starts a series of events or mechanisms that eventually lead to

the rise of PGF2α, followed by a decline of PGF2α several days later. The time delays are thus a 'black box' where the intermediate events that regulate PGF2α levels are not described. In this way, we were able to obtain the right time point of CL regression even though we do not know the biological mechanisms exactly. By reducing the delays, the duration of the luteal phase can be reduced. This could mean that P4 serum levels already decline during the second wave of follicle development, which could then become the ovulatory wave. The shorter delays could thus result in a shorter cycle with only 2 follicular waves. However, the consequence of the chosen approach is that the predictive abilities for this part of the model are limited. Model improvement and refinement of this sub-model will play an important role in future work.

Apart from fitting of the model to individual cow data, mentioned above, we plan to use this model to determine the level of control exerted by various system components on the functioning of the system. Examples of such model applications are to explore the mechanisms that influence the pattern of follicular waves, or to study hormone patterns associated with subfertility. Also, 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. Possible extensions of the model could be in the field of energy metabolism, stress, disease, and factors affecting the expression of estrous behavior. There are relationships between regulation of the estrous cycle and energy balance, which can cause fertility problems in high producing dairy cows in negative energy balance (for reviews see Diskin et al., 2003; Roche, 2006). Changes in reproductive performance that are associated with high milk production may in part be explained by elevated P4 and E2 clearance rates, as described in the physiological model of Wiltbank et al. (2006). In this physiological model, clearance rates of hormones by the liver of cows with high milk production are increased as a result of elevated feed intake, leading to an increased liver blood flow and metabolic activity. With a similar level of hormone production, circulating hormone levels would thus be lower. Lameness, an example of a stress inducing condition, was found to inhibit the LH surge and ovulation, whereas incidence of estrous behavior (although with less intensity) was not reduced. These observations suggest that stress, caused by lameness, reduces P4 exposure before estrus and/or E2 production by the dominant follicle (Dobson et al., 2008; Walker et al., 2008). Further, a normal endocrinological cycle is prerequisite for appropriate expression of estrous behavior. The relationships found between P4, E2 and intensity of estrous behavior show that hormones involved in regulation of the estrous cycle also affect the expression of estrous behavior (Lyimo et al., 2000; Roelofs et al., 2005). These and other findings and

hypotheses about regulation of the bovine estrous cycle could be translated into mathematical equations or modified parameterization and incorporated in the current model.

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4

Mechanisms regulating follicle wave patterns in the bovine estrous cycle investigated with a mathematical model

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Abstract

A normal bovine estrous cycle contains 2 or 3 waves of follicle development, and ovulation takes place in the last wave. However, the biological mechanisms that determine whether a cycle has 2 or 3 waves have not been elucidated. In a previous paper, we described a mathematical model of the bovine estrous cycle that generates cyclical fluctuations of hormones, follicles, and corpora lutea in estrous cycles of approximately 21 days for cows with a normal estrous cycle. The parameters in the model represent kinetic properties of the system with regard to synthesis, release, and clearance of hormones and growth and regression of follicles and corpora lutea. The initial model parameterization resulted in estrous cycles with 3 waves of follicular growth. Here, we use this model to explore which physiological mechanisms could affect the number of follicular waves. We hypothesized that some of the parameters related to follicle growth rate or to the time point of CL regression are likely candidates to affect the number of waves per cycle. We performed simulations with the model in which we varied the values of these parameters. We showed that variation of (combinations of) model parameters regulating follicle growth rate or time point of CL regression can change the model output from 3 to 2 waves of follicular growth in a cycle. In addition, alternating 2- and 3-wave cycles occurred. Some of the parameter changes seem to represent plausible biological mechanisms that could explain these follicular wave patterns. In conclusion, our simulations indicated likely parameters involved in the mechanisms that regulate the follicular wave pattern, and could thereby help to find causes of declined fertility in dairy cows.

Key words: mathematical model, estrous cycle, follicular wave pattern

4.1 Introduction

Two different patterns of follicle development are identified in mammals. In humans (and rats and pigs), the development of follicles to ovulatory size occurs only during the follicular phase, whereas in cattle (and sheep and horses), development of follicles to ovulatory or near-ovulatory size occurs throughout the cycle (Fortune, 1994). A normal bovine estrous cycle consists of 2 or 3 waves in which a cohort of follicles starts to grow. Many studies report mostly 2-wave cycles (Ahmad et al., 1997; Bleach et al., 2004; Wolfenson et al., 2004; Burns et al., 2005; Jaiswal et al., 2009). Cycles with 1 or 4 waves occur incidentally (Bleach et al., 2004; Wolfenson et al., 2004). In both 2- and 3-wave cycles, serum levels of FSH start to increase directly after ovulation (day 0), inducing the emergence of the first follicular wave. Typically, emergence of the second wave occurs on day 9 to 10 in 2-wave cycles and on day 8 to 9 in 3-wave cycles. In 3-wave cycles, a third wave emerges on day 15 to 16. The first 1 or 2 waves produce a dominant follicle that does not ovulate but undergoes regression under influence of P4. The functional dominant follicle present at the onset of luteolysis becomes the ovulatory follicle. Regression of the CL starts earlier in 2-wave cycles (day 16) than in 3-wave cycles (day 19), resulting in cycle lengths of 19 to 20 days and 22 to 23 days, respectively (reviewed by Adams et al., 2008).

The reason for cycles being of the 2- or 3-wave type is unclear. No difference was found between cows and heifers with regard to the proportion of 2- and 3-wave cycles (Wolfenson et al., 2004). The number of waves in a cycle does not appear to be affected by breed or age (reviewed by Adams et al., 2008). An increase in 3-wave patterns has been associated with poor nutrition and heat stress (reviewed by Adams et al., 2008). A higher milk yield was reported in cows with 2-wave cycles (Bleach et al., 2004). Several experiments have been performed to search for endocrine mechanisms underlying 2-wave versus 3-wave cycles. One possible explanation of a difference in number of waves is a difference in CL life span. The onset of CL regression occurs 2.5 days earlier in 2-wave than in 3-wave cycles (Ahmad et al., 1997; Jaiswal et al., 2009). Another possible explanation is that slowly growing dominant follicles delay the start of the next wave. Ovulatory follicles in 2-wave cycles have a lower growth rate than ovulatory follicles in 3-wave cycles (Bleach et al., 2004). Cauterization of the dominant follicle of the first wave (i.e., reduced E2 and Inh production) at day 3 or 5 of the cycle resulted in an FSH surge the day after cauterization (Adams et al., 1992) and an earlier emergence of the next wave (Ko et al., 1991). Cows with 3-wave cycles had lower serum FSH and Inh concentrations in non-ovulatory waves compared with cows with 2-wave cycles (Parker et al., 2003), and it was therefore suggested that the number of waves

during the cycle is affected by serum FSH and Inh concentrations. The latter is confirmed by the finding that immunization against Inh-A increased the number of follicular waves during a cycle (Medan et al., 2006).

Mathematical modeling of the bovine estrous cycle could help in understanding the dynamics of this complex biological system. Recently, we developed a mathematical model of the bovine estrous cycle (Boer et al., 2011b). The objective of this paper was to investigate which mechanisms could be likely candidates for regulation of the number of waves in the bovine estrous cycle, using this model. A better understanding of the endocrine mechanisms regulating follicle development could help to find causes of declined fertility in dairy cows.

4.2 Materials and methods

4.2.1 Parameterization of follicle wave patterns

In cattle, the functional follicle that is dominant at the time of CL regression develops to become the ovulatory follicle. We assumed that 2 mechanisms may exist by which follicle wave pattern can be influenced. One is the rate of follicle growth and the other is the time point of CL regression. The first mechanism might be induced by changing the effect of FSH or P4 on follicle growth, or by changing FSH or P4 synthesis, because follicle growth is stimulated by FSH and inhibited by P4 (Ko et al., 1991; Parker et al., 2003; Medan et al., 2006). The second mechanism; that is, the time point of CL regression, is expected to have an effect on the follicular wave pattern because 2-wave cycles can occur when the CL starts to regress at an earlier time point; for example, because of an earlier increase of PGF2α (Adams et al., 1992; Ahmad et al., 1997; Jaiswal et al., 2009). We selected 10 parameters in our model (Table 4.1), of which 7 relate to the first mechanism and 3 to the second mechanism, and we tested whether changing the value of these parameters affects the number of waves per cycle in the model simulations. The model was simulated for 120 days and can be simulated for as many days as required. For each of the 10 parameters, we performed simulations in which we changed the parameter with small steps for a range of values around the initial value. Results were evaluated by studying the figures of the model solution. We did not further increase or decrease the value when the solution continued to be irregular. The rationale for the expected effect of a parameter on either follicle growth rate or time point of CL regression is given in more detail in Section 4.3, together with the simulation results for that parameter.

Table 4.1 *Parameter description, initial parameter value, and hypothesized direction of change (increase or decrease) to alter a 3-wave in a 2-wave cycle for the 10 parameters that were tested for an effect on follicular wave pattern*[1].

Parameter no.	Parameter symbol[2]	Parameter explanation	Initial value	Chance
1	T_{FSH}^{Foll}	Threshold of FSH above which the stimulating effect on Foll is increased	1.44	↑
2	m_{FSH}^{Foll}	Max. Foll growth rate stimulated by FSH	0.70	↓
3	m_{P4}^{Foll}	Max. inhibition rate on Foll of P4	2.17	↓
4	T_{Foll}^{FSH}	Threshold of Foll above which the stimulating effect of FSH on Foll decreases (larger follicles become more sensitive to (i.e. less dependent on) FSH)	0.30	↓
5	c_{Foll}^{Inh}	Proportionality factor of Foll to Inh (the production of Inh is proportional to Foll)	4.33	↑
6	T_{Inh}^{FSH}	Threshold of Inh above which the inhibiting effect on FSH synthesis is increased	0.06	↓
7	m_{Inh}^{FSH}	Max. FSH synthesis rate in absence of Inh	1.46	↓
8	c_{CL}^{P4}	Proportionality factor of CL to P4 (the production of P4 is proportional to CL function)	0.50	↓
9	c_{P4}^{OTR}	Proportionality factor of P4 to OTR (the production of OTR is proportional to P4 serum levels)	0.87	↑
10	$T_{OTR}^{PGF2\alpha}$	Threshold of OTR above which the stimulating effect on PGF2α is increased	3.97	↓

[1] *Parameter values are on a relative scale to simplify parameter estimation.*
[2] *T denotes a threshold, m denotes a maximum, and c denotes a rate constant.*

A sensitivity analysis for the complete model has been performed with the techniques described in Deuflhard (2004) and Grah (2004). Sensitivities have been calculated with external numerical differentiation of the system at specified time points. These sensitivities were obtained as difference quotients depending on the solution components and parameter perturbations. The perturbations were taken as 0.1% of the absolute parameter values or at least $10e^{-5}$. The values were then weighted according to parameter ranges and measurement information to obtain the scaled sensitivity matrix. The column norms of the sensitivity matrix represent

the relative sensitivities of the system with regard to each parameter. A higher column norm indicates a parameter whose change in value has a larger effect on the model solution compared with changes of other parameter values, which means that this parameter is a more sensitive parameter.

4.2.2 Brief description of the model

Recently, we developed a deterministic mathematical model that describes the dynamics of the bovine estrous cycle as a set of linked differential equations, including the processes of follicle and CL development and the working of key hormones that interact to control these processes (Boer et al., 2011b). In the differential equations, Hill functions (Boer et al., 2011b) were used for the modeling of inhibitory and stimulatory effects of hormones. A Hill function is a sigmoidal function between 0 and 1, which switches at a specified threshold from one level to the other with a specified steepness. Time delays were incorporated when appropriate, to capture the time needed for factors to influence each other. Parameter estimation was based on experimental measurements available in the literature. The simulations of this model are in line with empirical knowledge. With the parameterization of Boer et al. (2011b), the model generates estrous cycles of 21 days, with 3 peaks of FSH and 3 corresponding waves of follicle growth and Inh production. In the model, *CL* denotes the CL function; that is, the capacity of the CL to produce P4, rather than the physical size of the CL. Likewise, follicular function (*Foll*) represents the combined capacity of all follicles present at any time to produce E2 and Inh. Each follicular wave is induced by an increase in FSH. Progesterone, which is high during the first 2 waves, decreases as the CL regresses under influence of PGF2α released from the uterus. The third wave of follicle growth then results in increasing levels of E2. This causes a surge of GnRH and hence LH, which triggers ovulation (Figures 4.1 and 4.2). More details about the incorporated physiological mechanisms and the mathematical description of the model can be found in Boer et al. (2011b).

4.2.3 Modification of the model

The model described in Boer et al. (2011b) includes 2 fixed time delays for the effect of the increase in P4 levels on PGF2α release, to mimic the situation that the increase of P4 early in the cycle starts a series of events or mechanisms that eventually leads to the increase of PGF2α, followed by a decline of PGF2α a few days later. The time delays are thus a “black box” in which the intermediate events that regulate PGF2α levels are not described. We have adapted this part of the

model. In the model used for the current study, these delays are replaced by a mechanism in which the ability to synthesize PGF2α develops over time under influence of P4. Exposure to P4 above a threshold level must last for a couple of days to induce PGF2α release. Progesterone stimulates the synthesis of receptors (e.g., OTR) and enzymes required for the production and release of PGF2α (Silvia et al., 1991; dos Santos et al., 2009). In the model, *OTR* thus represents the overall mechanism in the endometrium involved in the production of PGF2α; OTR is stimulated by P4,

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

where c_{P4}^{OTR} is the proportionality factor of P4 in the increase of OTR, c_{OTR} is the decrease rate of OTR, and (t) denotes time; OTR stimulates PGF2α,

$$\frac{d}{dt}PGF2\alpha(t) = H_{14}^{+}(OTR) - c_{PGF2\alpha} \cdot PGF2\alpha(t)$$

where $H^{+}(OTR)$ is the Hill function of the stimulating (+) effect of OTR, and $c_{PGF2\alpha}$ is the PGF2α clearance rate constant. Levels of PGF2α thus increase because P4 stimulates the production of OTR required for PGF2α production (Figures 4.1 and 4.2). Figure 4.1 shows an overview of the mechanisms incorporated in the current model. The equations and parameter values are listed in Appendix 2. The model contains 13 differential equations and 54 parameters.

4.3 Results and discussion

Of the 10 parameters tested, 6 affected the number of waves per cycle (Table 4.2). Cycles with 2 waves had a shorter cycle length. In the non-ovulatory wave of 2-wave cycles, FSH levels were higher, *Foll* (follicular capacity to produce E2 and Inh) was larger, and therefore E2 and Inh levels were also higher compared with non-ovulatory waves of 3-wave cycles. The 2-wave cycles obtained by a change in follicle growth rate were due to a later emergence of the second wave, whereas the 2-wave cycles obtained by a change in time point of CL regression were caused by a shorter CL life span.

4.3.1 Effects of parameters related to follicle growth rate

4.3.1.1 Effect of FSH. Follicle growth rate is stimulated by FSH, and the FSH-dependent growth rate in the model is influenced by several parameters: for example, maximum rate of FSH-dependent growth (m_{FSH}^{Foll}) and threshold of FSH to stimulate *Foll* (T_{FSH}^{Foll}). The parameters m_{FSH}^{Foll} and T_{FSH}^{Foll} belong to the Hill function of

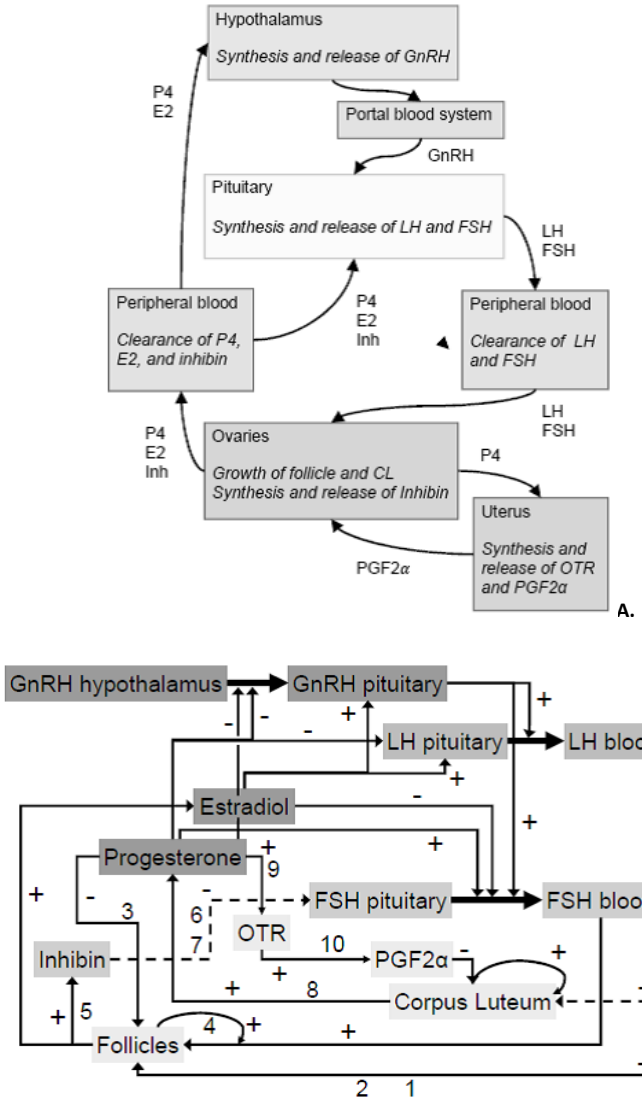

Figure 4.1 *(A) Compartmental representation of the basis for the mathematical model of the bovine estrous cycle. Each process is represented by a set of equations. (B) Mechanisms of the model of the bovine estrous cycle, in which the boxes represent the 13 components for which a differential equation was derived. Stimulating and inhibiting effects are denoted by '+' and '−', respectively; dashed lines denote a time delay. The numbers (as used in Table 4.1) of the parameters that were changed in the simulations of this study are placed at the mechanism (arrow) that they affect.*

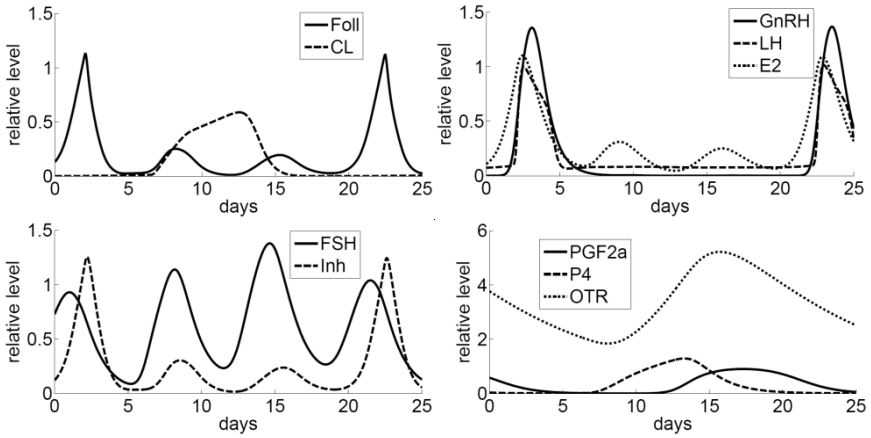

Figure 4.2 *The initial model parameterization results in estrous cycles with 3 waves of follicular growth. The equations are expressed on a relative scale to simplify parameter estimation, and therefore the y-axis of the figures is dimensionless.*

the effect of FSH on *Foll*, where the first represents the maximum FSH-dependent follicle growth rate, and the second defines the threshold of FSH at which the stimulatory effect on *Foll* increases. We tested whether a lower follicle growth rate could be simulated by a decrease in m_{FSH}^{Foll}. In the simulations, a decrease in m_{FSH}^{Foll} alone did not result in a 2-wave cycle, but the wave pattern could be changed in combination with another parameter involved in follicle growth rate, maximum inhibition rate of P4 on *Foll* (m_{P4}^{Foll}). A decrease in m_{FSH}^{Foll} from 0.70 to 0.40 resulted in 2-wave cycles when, at the same time, m_{P4}^{Foll} was decreased, and the resulting *Foll* peak height in non-ovulatory waves was higher (Figure 4.3). Apparently, despite the lower FSH-dependent growth rate, the first wave could then grow larger and persist for longer due to the lower inhibiting effect of P4. Therefore, the second wave occurred when the CL was already waning, allowing the second wave to become the ovulatory wave.

Remarkably, increasing m_{FSH}^{Foll} from 0.70 to 1.35 (without a change in m_{P4}^{Foll}) resulted in alternating 2-wave and 3-wave cycles (Figure 4.4). Increasing m_{FSH}^{Foll} even further, to 1.80, resulted in a series of 2-wave cycles. However, LH peaks then became irregular, which appeared to be related to the increased E2 levels produced by the larger follicles. Decreasing the sensitivity of the pituitary for E2 resulted in normal LH peaks again. This was done by increasing the E2 threshold for GnRH sensitivity of the pituitary ($T_{E2}^{GnRH,2}$), which is the threshold of E2 at which

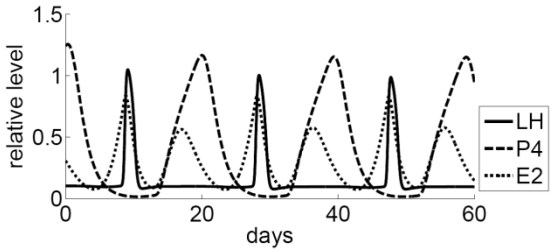

Figure 4.3 *A decreased maximum rate of FSH-dependent growth (m_{FSH}^{Foll}) combined with a decreased maximum inhibition rate of P4 on Foll (m_{P4}^{Foll}) resulted in cycles with 2 follicular waves. The equations are expressed on a relative scale to simplify parameter estimation, and therefore no dimension is given at the y-axis of the figures.*

the pituitary becomes more sensitive to GnRH. When $T_{E2}^{GnRH,2}$ was increased from 0.88 to 1.62, the GnRH/LH surge was induced at the appropriate time point; that is, together with E2 peak levels. Likely, an increase of m_{FSH}^{Foll} (instead of the expected decrease) results in a 2-wave cycle because it takes longer before the inhibiting effect of P4 becomes stronger than the increased stimulating effect of FSH, and therefore the first follicular wave starts to decline at a later time point. We tested whether a lower follicle growth rate could be simulated by an increase in T_{FSH}^{Foll}. However, a change in T_{FSH}^{Foll} did not affect wave pattern.

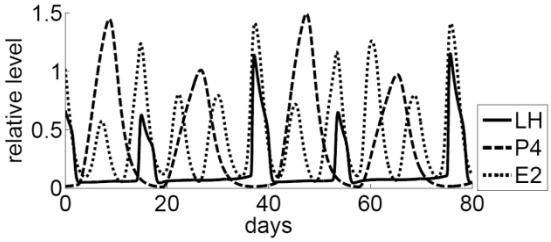

Figure 4.4 *When the maximum rate of FSH-dependent growth (m_{FSH}^{Foll}) was increased from 0.70 to 1.35, the model simulated alternately 2-wave and 3-wave cycles.*

In the model, larger follicles are less dependent on FSH; that is, follicles larger than the threshold T_{Foll}^{FSH} (threshold of *Foll* to downscale FSH threshold) become more sensitive to (i.e., less dependent on) FSH. Therefore, we tested whether a decrease in T_{Foll}^{FSH} would lead to fewer waves per cycle. Simulation results showed that this parameter indeed affected the wave pattern. When T_{Foll}^{FSH} was decreased from 0.30 to 0.15, the model simulated alternating 2-wave and 3-wave cycles, with some variation in hormone levels between cycles. When this

parameter was 0.12, almost all cycles had 2 waves (Figure 4.5). With values below 0.12, E2 and LH patterns became irregular.

4.3.1.2 Effect of P4. We assumed that when follicle growth is less inhibited by P4, follicles in the luteal phase could grow larger and persist for longer, which could result in a higher occurrence of cycles with 2 waves. We have tested this hypothesis by decreasing the maximum inhibition rate of P4 on *Foll* (m_{P4}^{Foll}), which is the parameter that represents the maximum inhibiting effect of P4 on follicular function. The model generated cycles with 2 waves when m_{P4}^{Foll} was between 0.60 and 1.20. When m_{P4}^{Foll} was between 1.20 and 1.80, the cycle contained sometimes 2 and sometimes 3 waves, or somewhat irregular patterns. When m_{P4}^{Foll} was 1.80 or higher, the model generated cycles with 3 waves, and the peak levels of *Foll* in non-ovulatory waves became lower with further increased m_{P4}^{Foll} (because the inhibiting effect of P4 became stronger) until the cycle contained basically 1 ovulatory wave, without waves during the luteal phase.

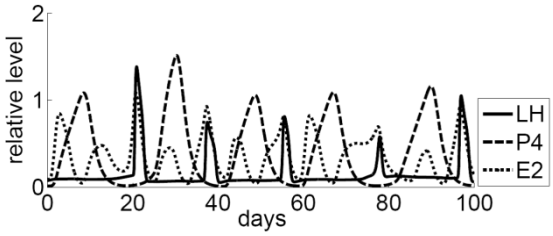

Figure 4.5 *When the threshold of Foll to downscale FSH threshold (T_{Foll}^{FSH}) was 0.12, almost all cycles had 2 waves.*

4.3.1.3 FSH Synthesis. Another possible mechanism that may affect follicle growth rate is FSH synthesis. We assumed that a stronger inhibiting effect of Inh on FSH synthesis could result in lower FSH serum levels and therefore slower follicle growth, resulting in a cycle with 2 instead of 3 waves. We tested whether a decrease in maximum FSH synthesis rate in the pituitary (m_{Inh}^{FSH}) could reduce the FSH-dependent follicle growth rate and result in 2-wave cycles. However, a decrease in this parameter did not result in a regular 2-wave cycle, but in low peak levels of *Foll* without LH surge. This was due to E2 levels that were too low to induce the GnRH/LH surge. The E2-dependent GnRH/LH surge could not be repaired by a decrease in E2 threshold for GnRH sensitivity of the pituitary ($T_{E2}^{GnRH,2}$). We also tested whether a decrease in the threshold of Inh for inhibition of FSH synthesis (T_{Inh}^{FSH}), which results in a stronger inhibition of FSH synthesis by Inh, could result in a cycle with 2 waves. However, a decrease in T_{Inh}^{FSH} did not

change the pattern of 3 waves, and below 0.03, no LH surges occurred. Although a large increase in T_{Inh}^{FSH} resulted in a changed wave pattern, FSH and Inh levels became irregular such that we did not consider this as a normal 2-wave cycle.

In the model, the production of Inh was assumed to be proportional to *Foll* with a short delay, where c_{Foll}^{Inh} is the proportionality factor of *Foll* to Inh. We assumed that FSH synthesis would be reduced when Inh production by the follicles was higher. Therefore, we tested whether an increase in c_{Foll}^{Inh} would increase circulating Inh levels and result in a cycle with 2 waves, but this was not found.

Table 4.2 *Overview of whether a change in parameter value affects the number of waves per cycle and of the changes in parameter values that resulted in a different number of waves.*

Parameter symbol[1]	Change in wave number	Initial value[2]	Adapted value	Number of waves	Remark
T_{FSH}^{Foll}	No	1.44			
m_{FSH}^{Foll}	Yes	0.70	0.40	2	When m_{P4}^{Foll} is 0.50
			1.80	2	When $T_{E2}^{GnRH,2}$ is increased from 0.88 to 1.62
m_{P4}^{Foll}	Yes	2.17	1.00	2	
			1.45	2 and 3[3]	
T_{Foll}^{FSH}	Yes	0.30	0.12	2	Almost all cycles 2 waves
c_{Foll}^{Inh}	No	4.33			
T_{Inh}^{FSH}	Yes	0.06	0.30	2	Irregular FSH and Inh levels
m_{Inh}^{FSH}	No	1.46			
c_{CL}^{P4}	No	0.50	1.45	3	when m_{P4}^{Foll} is 1.00
c_{P4}^{OTR}	Yes	0.87	2.00	2	
$T_{OTR}^{PGF2\alpha}$	Yes	3.97	2.00	2	
			2.50	2 and 3[3]	

[1] *See Table 4.1 for definition of parameters*

[2] *The initial parameter value results in 3-wave cycles*

[3] *Two- and 3-wave cycles alternating*

4.3.1.4 P4 Synthesis. Another mechanism we tested was the inhibiting effect of P4 on follicle growth rate. We assumed that a 2-wave cycle could occur when a follicle wave can grow for longer due to less inhibition by P4. A reduced inhibitory effect on follicle growth rate could be due to a lower P4 production by the CL, resulting in lower P4 serum levels. In the model, it is assumed that P4 is proportional to CL

function. Therefore, we tested whether a decrease of proportionality factor of CL to P4 (c_{CL}^{P4}) would affect P4 levels and follicle growth rate. Simulation results did not show a clear effect of P4 levels on the time course of a follicular wave. A decrease in c_{CL}^{P4} resulted in lower peak P4 levels, but did not result in a 2-wave cycle. However, a decrease in c_{CL}^{P4} combined with a decrease in m_{P4}^{Foll} resulted in an interesting wave pattern. As described earlier, reduced inhibition of follicle growth rate by P4 (by reducing m_{P4}^{Foll}) resulted in a 2-wave cycle when P4 serum levels were not changed. However, when P4 serum levels were reduced, by decreasing c_{CL}^{P4} from 1.45 to 0.50, the wave pattern changed back to 3 waves (Figure 4.6). Intermediate values for c_{CL}^{P4} (between 0.50 and 1.45, in combination with m_{P4}^{Foll} decreased to 1.00) resulted in cycles with sometimes 3, sometimes 2 waves. Thus, a parameter set was identified in which 2 parameter values were changed compared with the initial set that also simulates estrous cycles with 3 waves, but with different values for some of the output functions. In particular, E2 peak levels during non-ovulatory waves in this new combination of parameter values for a 3-wave cycle were higher than in the initial model parameterization for 3-wave cycles, possibly because of the reduced sensitivity of the follicles for P4. Further, the cycle length was a few days longer, probably because the lower P4 serum levels caused a later increase in OTR and thus CL regression. Therefore, a third wave could develop.

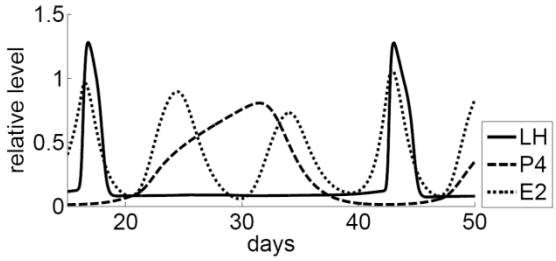

Figure 4.6 *Three-wave cycle when the proportionality factor of CL to P4 (c_{CL}^{P4}) = 0.5 and the maximum inhibition rate of P4 on Foll (m_{P4}^{Foll}) = 1.0.*

4.3.2 Effects of parameters related to time point of CL regression

Besides the effect of follicle growth rate, we expected that the time point of CL regression would have an effect on the follicular wave pattern. Earlier CL regression could be due to an earlier increase of PGF2α. Therefore, we tested whether a decreased threshold of OTR to stimulate PGF2α increase ($T_{OTR}^{PGF2\alpha}$) could result in PGF2α release at a lower OTR concentration and thus at an earlier time point, resulting in a cycle with 2 follicular waves. As expected, the simulation results

showed that a change in time point of CL regression had a large effect on the number of follicular waves. A noteworthy result was that varying $T_{OTR}^{PGF2\alpha}$ could result in 1, 2, 3, or 4 waves in a cycle (Table 4.3), because the time point of PGF2α increase was changed. When the number of waves in a cycle increased, cycle length became longer and P4 levels higher, whereas E2 levels in the luteal phase became lower.

We tested whether a stronger P4 stimulation of OTR could also be obtained by an increased proportionality factor of P4 to OTR (c_{P4}^{OTR}). An increase in c_{P4}^{OTR} from 0.87 to 2.00 resulted in a cycle with 2 waves (Figure 4.7). The higher proportionality factor resulted in higher levels of OTR and lower levels of P4 compared with 3-wave cycles. An overview of the differences in parameter values between 2-wave and 3-wave cycles is given in Table 4.2.

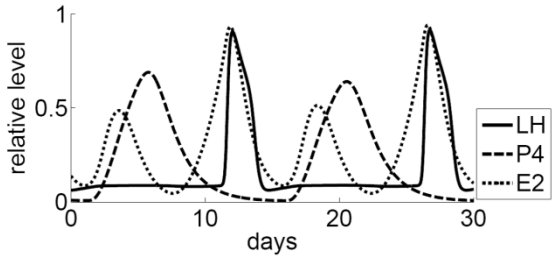

Figure 4.7 *An increase in the proportionality factor of P4 to OTR (c_{P4}^{OTR}) resulted in a cycle with 2 waves.*

4.3.3 Sensitivity analysis

The sensitivity analysis showed that the 3 parameters that easily induced a change in wave number ($T_{OTR}^{PGF2\alpha}, c_{P4}^{OTR}, and\ m_{P4}^{Foll}$) were the most sensitive in the group of the 10 tested parameters. The parameters that did not induce a change in wave number had the lowest sensitivity in the group of tested parameters, whereas the parameters that needed an additional change in another parameter (m_{FSH}^{Foll}) or did not always result in a 2-wave cycle (T_{Foll}^{FSH}) had an intermediate sensitivity.

Table 4.3 *Overview of variations in the value of the threshold of OTR above which the stimulating effect on PGF2α is increased ($T_{OTR}^{PGF2\alpha}$) and the according number of waves per estrous cycle.*

Value of $T_{OTR}^{PGF2\alpha}$	Number of follicular waves
0.25	1
0.70	1 and 2 alternating
2.00	2
2.50	2 and 3 alternating
4.10	3
7.50	3 and 4 alternating
9.10	4

4.3.4 General discussion

We used a mathematical model of the bovine estrous cycle to identify critical points in the mechanisms that affect the number of follicular waves in a cycle. We found that the model output changed from 3 to 2 waves in a cycle when the duration of the luteal phase was changed or when the effect of FSH or P4 on follicle growth was changed, but not when FSH synthesis or P4 synthesis was changed. Although some parameter changes resulted in irregular patterns, few simulations occurred in which the system was totally derailing or collapsing, and most of the times it recovered to a normal cycle, emphasizing the robustness of the model. Some parameterizations resulted in strictly periodic behavior of the cycle, whereas others resulted in quasiperiodic behavior. When a cycle had 2 waves instead of 3, the duration of the cycle was shorter, which is in line with the empirical observations reported by Jaiswal et al. (2009), Ahmad et al. (1997), and Adams et al. (2008). The sensitivity analysis confirmed that parameters that affected the pattern of follicular waves indeed had a strong effect on the model solution.

Depending on the parameterization, the model simulations showed the same wave pattern repeatedly in successive cycles or alternating 2- and 3-wave cycles. In the literature, the repeatability of wave pattern within individual cows is studied in a limited number of papers. It was shown that wave pattern is repeatable within individuals (Jaiswal et al., 2009) but also that cows can switch between cycles with 2 and 3 waves (Price and Carrière, 2004). Sichtar et al. (2010) reported an almost equal proportion of alternating and nonalternating patterns in cows monitored during 3 cycles. In a study of Rhodes et al. (1995) in 5 *Bos indicus* heifers monitored for at least 12 consecutive estrous cycles, 3 waves was the most common pattern. However, none of the cows showed 3 waves in all cycles; cycles with 2 and some with 4 waves were observed. Although the number of animals and consecutive cycles are limited, these studies suggest that both genetic and environmental factors may play a role in the regulation of follicular wave pattern.

For several single parameters, a shift in parameter value resulted in a cycle with 2 follicular waves instead of 3. Although sometimes regular 2-wave and 3-wave cycles could be simulated for intermediate values, other values resulted in more irregular patterns, especially for parameters related to follicle growth rate. Possibly, slight changes in other parameters are required to “correct” for these irregularities, which indeed occurs in cows. This required change in other parameters could be demonstrated by the change in m_{FSH}^{Foll}. An increase in m_{FSH}^{Foll} combined with an increase in $T_{E2}^{GnRH,2}$, or a decrease in m_{FSH}^{Foll} combined with a decrease in m_{P4}^{Foll} resulted in a 2-wave cycle.

A decrease in c_{CL}^{P4} alone did not change the number of waves, although P4 levels decreased. This could indicate that, for the regulation of the follicular wave pattern, the period in which P4 is produced is more important than the amount of P4 production (as long as P4 is above a minimum level). The simulation results clearly showed a gradual shift in the number of waves when $T_{OTR}^{PGF2\alpha}$ was changed. The time course of CL development and regression thus appears to have a distinct effect on the pattern of follicular waves, which is in line with the results of Jaiswal et al. (2009) who reported an earlier onset of CL regression in 2-wave cycles compared with 3-wave cycles.

No regular 2-wave cycles could be simulated by changing parameters related to the effect of Inh on FSH synthesis rate. However, the simulation results showed that 2-wave cycles had higher Inh and FSH levels than 3-wave cycles, which was also reported by Parker et al. (2003). However, doubling the amplitude of the FSH surge preceding the emergence of a follicular wave did not change the characteristics of that wave (Duggavathi et al., 2005). This suggests that FSH is normally not a limiting factor in follicle growth rate, but that changed Inh and FSH levels are a result rather than a cause of a changed follicular wave pattern. Although *Foll* in the model represents the capacity to produce E2 and Inh, it is correlated to follicle diameter. The higher levels of *Foll* in 2-wave cycles compared with 3-wave cycles in the model output are therefore in line with the larger size of dominant follicles in 2-wave cycles compared with 3-wave cycles reported by Townson et al. (2002) and Wolfenson et al. (2004). The later emergence of the second wave in 2-wave cycles compared with the initial 3-wave cycle in the model is in line with the reports of Jaiswal et al. (2009) and Adams et al. (1992).

For parameters related to follicle growth rate, it was difficult to induce a smooth shift from 3 to 2 waves, because irregular patterns appeared in between. In contrast, for parameters related to the time point of CL regression, this gradual switch resulted less often in irregular patterns. These simulation results are in line with empirical observations in the literature reporting that the number of follicular waves can be manipulated easily by changing the time point of CL regression (Diskin et al., 2002). In this way, the third wave does not fit anymore within the amount of time. It is not surprising that an earlier time point of CL regression (and therefore a shorter cycle) induces a switch from 3 to 2 waves without irregular patterns in between, because when P4 levels are sufficiently decreased at the second wave, this will become the ovulatory wave. Although in the bovine, 2-wave cycles are on average shorter than 3-wave cycles (Bleach et al., 2004; Adams et al., 2008), the difference is not equivalent to the duration of a complete wave. Based on reported differences in follicle development (Bleach et al., 2004; Jaiswal et al.,

2009), we postulate that differences in number of waves in natural estrous cycles may be due rather to changes in the mechanisms regulating follicle growth rate, and that the shorter cycle length is the result, not the cause, of the change in wave pattern. This is also shown by the simulation results because a change in follicle growth rate related parameters, resulting in 2-wave cycles, led to a shorter cycle length.

4.4 Conclusions

The simulation results showed that several components of our model of the bovine estrous cycle could affect the pattern of follicle growth. The number of waves could be affected by follicle growth rate as well as time point of CL regression. The simulation results suggest that the initial number of waves per cycle is determined by certain characteristics of follicle growth rate, and indicates likely parameters that are involved in this mechanism. A better understanding of the endocrine mechanisms regulating follicle development is important to obtain more precise control of the estrous cycle, which could help to improve pregnancy rates (Diskin et al., 2002). Experimental data to verify the predicted causes of 2- or 3-wave cycles are not always available, but the present simulation results show some likely candidates involved in the regulation of follicle wave pattern that could be tested in future experiments. These experiments require daily measurements of follicle and CL dynamics and blood sampling for successive cycles. Additionally, P4 or FSH could be administered to measure specific effects. Designated experiments are extensive, but the present simulation results show some candidates to consider for deeper investigation. Therefore, our results allow experimental effort to be focused on these candidates.

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5

Candidate mechanisms underlying atypical progesterone profiles as deduced from parameter perturbations in a mathematical model of the bovine estrous cycle

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Abstract

The complex interplay of physiological factors that underlies fertility in dairy cows was investigated using a mechanistic mathematical model of the dynamics of the bovine estrous cycle. The model simulates the processes of follicle and CL development and its relations with key hormones that interact to control these processes. Several factors may perturb the regular oscillatory behavior of a normal estrous cycle, and such perturbations are likely the effect of simultaneous changes in multiple parameters. The objective of this paper was to investigate how multiple parameter perturbation changes the behavior of the estrous cycle model, so as to identify biological mechanisms that could play a role in the development of cystic ovaries. Cystic ovaries are a common reason for reproductive failure in dairy cows, but much about the causes of this disorder remains unknown. We investigated in which region of the parameter space the model predicts a normal cycle, and when a progesterone pattern occurred with delayed ovulation (indicating a cystic follicle) or delayed luteolysis (indicating a persistent CL). Perturbation of the initial values for all parameters simultaneously showed 2 specific parameter configurations leading to delayed ovulation or delayed luteolysis immediately. The most important parameter changes in these 2 configurations involve the regulation of CL functioning, luteolytic signals, and GnRH synthesis, suggesting that these mechanisms are likely involved in the development of cystic ovaries. In the multidimensional parameter space, areas exist in which the parameter configurations resulted in normal cycles. These areas may be separated by areas in which irregular cycle patterns occurred. These irregular patterns thus mark the transition from one stable (normal) situation to another. Interestingly, within a series, there were some cycles with delayed ovulation and some with delayed luteolysis in these patterns. This could represent a situation of resumption of normal cyclicity (e.g., after parturition). In conclusion, the method of parameter perturbation used in the present study is an effective tool to find parameter configurations that lead to progesterone profiles associated with delayed ovulation and delayed luteolysis. Thereby, the model helps to generate hypotheses regarding the underlying cause of the development of cystic ovaries, which could be investigated in future experiments.

Key words: mathematical model , estrous cycle , progesterone, cystic ovaries

5.1 Introduction

Recently, we developed a deterministic mathematical model that describes the dynamics of the bovine estrous cycle as a set of linked differential equations. The model generates cyclical fluctuations of hormones, follicles, and corpora lutea in estrous cycles of approximately 21 days for cows with a normal estrous cycle. The parameters in the model represent kinetic properties of the system with regard to synthesis, release, and clearance of hormones, and growth and regression of follicles and corpora lutea (Boer et al., 2011b). Model simulations are in line with empirical observations, indicating that the model is a credible representation of reproductive physiology.

Several factors may disturb the regular oscillatory nature of the bovine estrous cycle. In the model, this translates to the question how much the parameter values could be perturbed without a change in the qualitative behavior of the model. This is interesting to assess, as it could identify biological mechanisms that are critical in the regulation of the bovine estrous cycle. The effects of changing specific parameters one at a time on the behavior of the model of the bovine estrous cycle was analyzed in Boer et al. (2011a), but in the reality of biological systems it is more likely that a perturbation is the effect of simultaneous changes in multiple physiological parameters, as can be simulated in the model by multi-parameter perturbations.

Several existing methods for multi-parameter robustness analysis (i.e., multi-dimensional sensitivity analysis; reviewed in Ghaemi et al., 2009) suffer from computational limitations because of the exponential increase of the number of combinations in systematic variation of multiple parameters. Here, we use an approach that is especially designed to be efficient for systems with a large number of parameters (Apri et al., 2010). More specifically, we wanted to identify parameters that are involved in the formation of cystic ovaries, an important cause of reproductive failure in dairy cows (Garverick, 1997; Braw-Tal et al., 2009). Cystic follicles or persistent corpora lutea develop when the dominant follicle or the CL fails to regress and maintains steroidogenesis. Metabolic and endocrine adaptations to high milk yield and negative energy balance are known to increase the risk of the formation of cystic ovaries, but the exact pathogenesis is unclear (reviewed in Vanholder et al., 2006). Identification of parameter perturbations involved in the formation of cystic ovaries could help in understanding which biological mechanisms play a role. Likely, there is not one exact pathogenesis, but different configurations of unfavorable physiological settings, resulting in cystic follicles or persistent corpora lutea. The modeling approach used here allows identification of different candidate scenarios.

The objective of this paper was to assess how physiological parameters may disturb the regular oscillatory nature of the bovine estrous cycle, and to identify mechanisms that could play a role in the formation of cystic ovaries. To obtain this, the P4 profiles simulated by the model were evaluated on the occurrence of delayed ovulation, indicating a cystic follicle, or delayed luteolysis, indicating a persistent CL.

5.2 Materials and methods

Our approach to search for mechanisms that could play a role in the formation of cystic ovaries can be summarized as follows. Using a mechanistic mathematical model of the bovine estrous cycle, we investigated in which region in the parameter space a normal cycle is simulated and when a P4 pattern associated with delayed luteolysis or delayed ovulation occurred. We defined 4 criteria for P4 profiles, as generated by the model, to categorize estrous cycles as normal or abnormal. By perturbing the initial values for all 60 parameters simultaneously, the parameter space was then scanned to detect where a normal cycle is predicted and where a pattern of delayed ovulation or delayed luteolysis occurs. By doing so, we obtained an estimation of the robustness region of the model (i.e., the whole region of parameter values for which a normal cycle is predicted). The model, the method of parameter perturbation, and the criteria for a P4 profile of a normal estrous cycle are described in more detail in the next paragraphs.

5.2.1 Brief description of the model

We used the deterministic mathematical model of the bovine estrous cycle originally described by Boer et al. (2011a) and Boer et al. (2011b) with the modifications introduced more recently by Stötzel et al. (2012). The model describes the dynamics of the bovine estrous cycle as a set of linked differential equations, including the process of follicle and CL development and the relations with key hormones that interact to control these processes (Figure 5.1). In the model, follicles are not modeled separately, and *Foll* represents the capacity of follicles present at any moment to produce estradiol (E2) and inhibin, rather than follicular size. Likewise, CL represents the capacity of the CL to produce P4. The model generates estrous cycles of approximately 21 days, with 2 or 3 peaks (depending on the parameterization) of FSH and 2 or 3 corresponding waves of follicle growth and, hence, E2 and Inh production. Progesterone, which is high during the first wave(s) of the cycle, decreases as the CL regresses under influence of PGF2α release from the uterus. Prostaglandin F2α is stimulated by OT and OTR (representing the overall OT-mediated mechanism in the endometrium involved in

the production of PGF2α). The effect of PGF2α on the CL is mediated by several local factors, such as endothelin-1-system, cytokines, and nitric oxide, included in the model as interovarian factors. When the CL has regressed, the last follicular wave results in increasing E2 levels. This causes a surge of GnRH and hence LH, which triggers ovulation. The part of *Foll* regressing due to the LH surge (i.e., the ovulated follicle) is preserved in the system and forms the developing CL in the next estrous cycle. The model contains 15 differential equations (Figure 5.1) with 60 parameters. The initial parameterization (default settings) results in estrous cycles with 3 waves of follicle growth (Figure 5.2). A complete overview of all equations and initial parameter values can be found in Appendix 3.

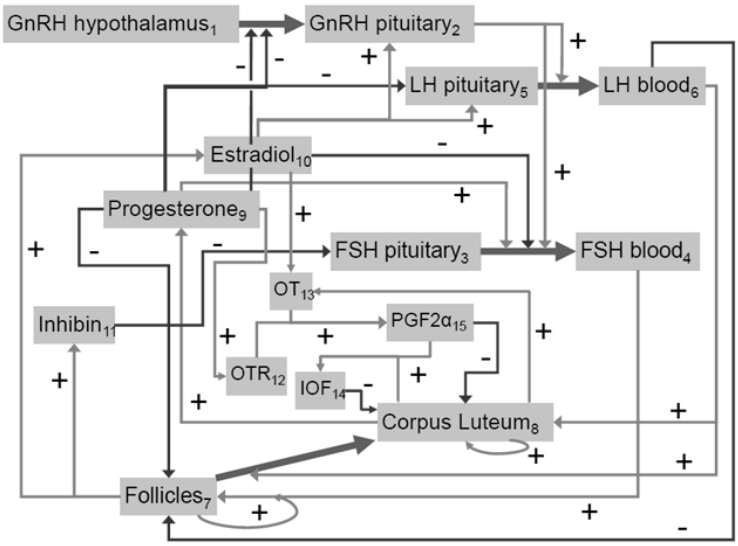

Figure 5.1 *Mechanisms of the model of the bovine estrous cycle, in which the boxes represent the 15 components for which a differential equation was derived. The numbers in the subscripts indicate the equations (Appendix 3A) that belong to each component. At the arrows (indicating specific parameterizations of a given equation), stimulating and inhibiting effects are denoted by + and −, respectively.*

5.2.2 Estimation of the robustness region

The initial values (i.e., default settings or baseline values) of the 60 model parameters (the so-called nominal set, given in Appendix 3C) result in a normal periodic solution with 3 waves of follicle development per cycle (Figure 5.2). However, it is expected that in a certain region in the 60-dimensional space around this nominal set the system still shows the oscillatory behavior that is characteristic for a normal cycle. We refer to this region as the robustness region. The model

parameters differ in sensitivity, and this oscillatory behavior is more easily perturbed by some parameter combinations than by others. We assume that this represents that certain parts of the biological system are more sensitive to environmental or genetic changes than others. Using criteria for a normal estrous cycle (as defined in the next section), the method described in Apri et al. (2010) was adjusted and applied to find an approximation of the robustness region. The present method is highly efficient for systems with a high-dimensional parameter space, as the number of simulations scales linearly and not exponentially with the number of parameters.

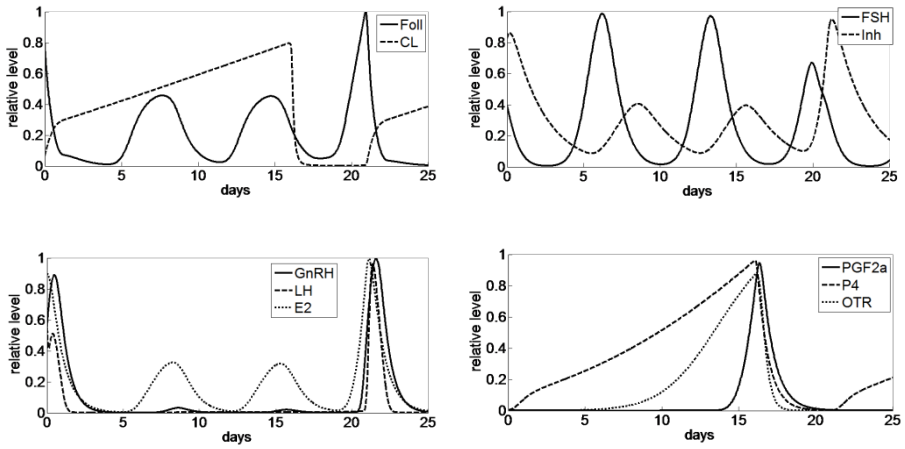

Figure 5.2 *The initial model parameterization (default settings) results in estrous cycles with 3 waves of follicular growth. The equations are expressed on a relative scale to simplify parameter estimation, and therefore, the y-axis of the figures is dimensionless.*

The adjusted method can be explained as follows. First, a gradient vector, ∇g, of which the entries represent the sensitivity of luteal phase length, g (as defined in the next section), to changes in the parameter values, k, was calculated according to equation 24 in Apri et al. (2010):

$$\nabla g = \left(\frac{\partial g}{\partial k_1}, \ldots, \frac{\partial g}{\partial k_m}\right).$$

This gradient vector is calculated numerically using

$$\frac{\partial g}{\partial k_j} \approx \frac{g(k_1,\ldots,k_j+\varepsilon,\ldots,k_m)-g(k_1,\ldots,k_m)}{\varepsilon}, j = 1, \ldots, m,$$

in which ε (a small positive number) is made smaller and smaller until convergence is reached. Here, the function $g(k1, \ldots, km)$ represents the luteal phase length as a

function of the parameter set k = (*k1*, . . . , *km*). This gradient vector was normalized by defining the sensitivity vectors:

$$S_j = \frac{\partial g}{\partial k_j}\frac{k_j}{g}, \qquad j = 1, \dots, m.$$

The advantage of this normalization is that it yields a dimensionless sensitivity vector S = (*S1*, *S2*, . . . , *Sm*). This sensitivity vector represents the direction in which the luteal phase length changes most. Next, other perturbation directions were constructed such that they are not only perpendicular to the first direction S, but also perpendicular to each other. These other perturbation directions are calculated by the Gram-Schmidt method. The whole procedure leads to, in total, 60 vectors, which are all mutually perpendicular and include the sensitivity vector S. However, to get a first impression of the robustness region, initially a 2-dimensional robustness analysis was carried out for the 2 parameters to which the luteal phase length was most sensitive. Then, starting from the nominal set (k_0), all parameter values were perturbed in the directions of these perpendicular vectors to detect where along these directions the model behavior shows a qualitative transition. For each vector, one may move in 2 opposite directions, so in fact one may deviate from the nominal set (k_0) in 120 directions. Note that if one moves in one of those directions, the values of all 60 parameters may change simultaneously, as these directions are not parallel to the parameter axes. The perturbation along a certain direction was stopped if one of the parameters approached zero (because all parameters should be positive), if P4 levels became too high (as defined in the next section), or if the P4 profiles met the definition for delayed ovulation or delayed luteolysis (see also next section). The model was simulated for 10 successive luteal phases.

5.2.3 Definition of progesterone profiles for a normal cycle

A normal estrous cycle consists of a period of elevated P4 levels (luteal phase) of about 15 days and a period of low P4 levels (inter-luteal interval or estrous phase) of about 6 days (Darwash et al., 1998). A cystic follicle or persistent CL occurs when the follicle or CL, respectively, fails to regress and persists on the ovary. Consequently, alterations in hormone patterns occur. Delayed ovulation involves the formation of a cystic follicle, leading to a prolonged interluteal interval (i.e., an extended period of low P4 serum levels). Delayed luteolysis involves the formation of a persistent CL, leading to a prolonged luteal phase length (i.e., an extended period of high P4 serum levels). Both abnormalities are thus represented by atypical P4 patterns. Abnormal P4 profiles were classified by Darwash et al. (1998),

and the classification has subsequently been applied by others (Garmo et al., 2009; Petersson et al., 2006; Vanholder, 2005). We used these classifications to define when delayed ovulation and delayed luteolysis occur in the model when we perturb the model parameter values (Figure 5.3). The relative threshold levels used in the model can be linked to the levels reported by Darwash et al. (1998). In brief, the 4 criteria for a normal estrous cycle are 1) values >0 for all parameters, 2) maximum relative P4 levels below 2.00, 3) luteal phase length of 9 to 19 days, and 4) interluteal interval of maximum 12 days. Delayed ovulation in the model is defined as P4 concentration below the relative level 0.15 (where the peak luteal P4 level in the model is initially about 1.00) for more than 12 days. Delayed luteolysis in the model is defined as P4 concentration above the relative level 0.15 for more than 19 days. Simulations with the model (not presented) show that an estrous cycle has only 2 waves when P4 levels are above 0.15 for 9 days (resulting in a cycle length of 17 days), and this was taken as minimum duration of the luteal phase. Upper boundary for P4 levels was set at 2.00, which represents the maximum P4 levels reported by Darwash et al. (1998). Consequently, a cycle is considered normal when P4 levels are between 0.15 and 2.00 for 9 to 19 days in the luteal phase and below 0.15 for less than 12 days in the interluteal interval. With these definitions, the initial length of the interluteal interval and the luteal phase length in the model are similar to the mean lengths in Darwash et al. (1998). Whether the model predicts a normal cycle or not was calculated based on P4 levels only, but we also evaluated the model solution for E2 and LH to see if these plots were in line with the decision made by the model based on P4. Thus, a quantitative validation of model performance was done with P4 levels against Darwash et al. (1998), and a visual assessment of E2 and LH was done as intuitive confirmation of the results.

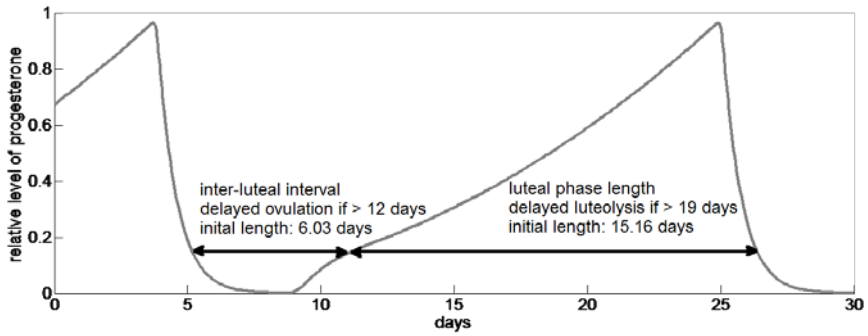

Figure 5.3 *Illustration of the luteal phase parameters analyzed in the model. The figure shows the P4 levels at the initial parameterization (nominal set).*

5.3 Results

5.3.1 Robustness region for a normal estrous cycle

The sensitivity analysis showed that the most sensitive parameter with respect to luteal phase length was parameter 41 (maximum increase of CL stimulated by itself), followed by parameter 51 (maximum increase of OTR stimulated by P4), as a change in these parameters induced the largest change in luteal phase length. To get a first impression of the robustness region, a 2-dimensional robustness analysis was carried out in the plane spanned by these 2 most sensitive parameters in the system. The cross-section of the robustness region within this plane is presented in Figure 5.4. This cross-section, colored in gray, was obtained by scanning the parameter space (k_{41}, k_{51}), while fixing the other parameters at their nominal value.

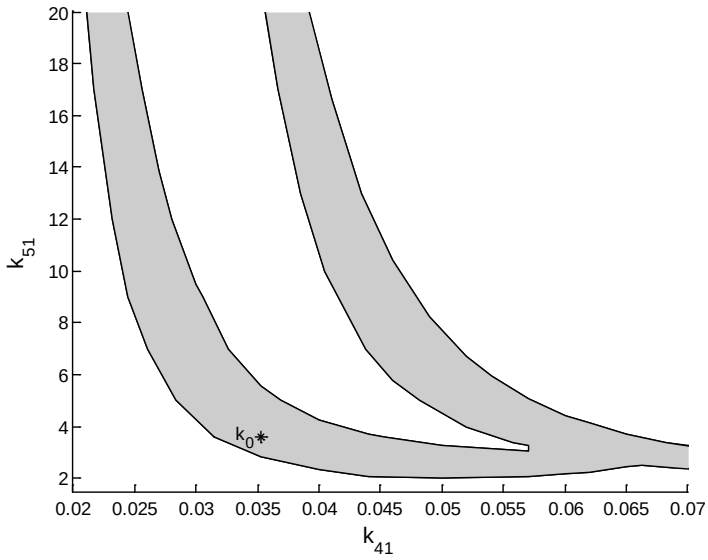

Figure 5.4 *The cross-section of robustness region of the model in the 2-dimensional (k_{41}, k_{51}) plane (gray area) for parameters 41 and 51. The nominal parameter set k_0 is marked by (*). A normal cycle is obtained when (k_{41}, k_{51}) take values in the gray area, keeping the other parameters fixed at nominal values. Note that the scale for k_{41} and k_{51} is not the same.*

For all points in the gray region the model predicts a normal cycle. Obviously, the nominal parameter set k_0 lies inside the robustness region. From Figure 5.4 it can be observed that the robustness region of the model might be quite complicated. Its cross-section with the (k_{41}, k_{51}) plane shows 2 distinct branches where a normal estrous cycle is predicted, and between the branches the model predicts a pattern of delayed ovulation or delayed luteolysis. The nominal parameter set k0 is situated

in the lower branch, close to the boundary of the robustness region. Because here only 2 parameters are allowed to vary at the same time, it is relatively easy to capture the exact shape of this part of the robustness region. However, if all parameters are allowed to vary simultaneously, a more effective way to estimate the robustness region is required.

The analysis was continued by estimating the 60-dimensional robustness region applying the method described in the Methods section. All perturbations were started from the nominal set (k_0). For convenience, all perturbation direction vectors were scaled such that each of them has 1 unit length. To give an impression of these vectors, the components of perturbation directions 1, 37, 41, and 53 are shown in Figure 5.5. As can be seen from this figure, in direction 1, components 41, 45, 51, and 52 have a relatively high value. Thus, in direction 1, parameters 41 (maximum increase of CL stimulated by itself), 45 (proportionality factor of CL in P4 increase), 51 (maximum increase of OTR stimulated by P4), and 52 (threshold of P4 to stimulate OTR increase) are more perturbed than the others. In direction 41, components 1 and 41 are dominant; thus, parameter 1 (synthesis rate constant of GnRH in the hypothalamus) and 41 are perturbed most. In all other perturbation directions, only 1 component is dominating. For each perturbation direction, the maximal variations are given, both in positive and negative directions, for which the system is still robust (i.e., for which the model predicts a normal cycle; Figure 5.6). For the 120 perturbation directions (up or down for 60 parameters), it occurred 9 times that the perturbation was stopped because one of the parameters passed zero, 6 times that P4 crossed the upper boundary, 10 times that delayed luteolysis was detected, 45 times that delayed ovulation was detected, and 50 times that delayed luteolysis as well as delayed ovulation occurred in a series of successive cycles (Figure 5.6). The minimum duration (9 days) of the luteal phase was never a reason to stop the perturbation. The plots of E2 and LH were always in line with the plot of P4 at the boundary of the robustness region. From Figure 5.6, it can be noticed that the robustness region is narrowest along the directions 1 and 41. In Figure 5.5, it is shown that direction 1 is dominated by parameters 41, 45, 51, and 52, so the system is very sensitive to simultaneous changes in these parameters. A similar conclusion holds for direction 41, which is dominated by parameters 1 and 41. Thus, 2 scenarios were found where the system becomes nonrobust immediately: for the first scenario (in direction 1), parameters 41, 45, 51, and 52 should be dominating in the perturbation, and for the second scenario (in direction 41), parameters 1 and 41 should be dominating in the perturbation (Table 5.1). In all other perturbation directions, only 1 parameter is dominating in the perturbation, but it is important to realize that these directions cannot be

considered as single parameter changes because the transition in qualitative behavior of the model is also due to the slight changes in other parameter values. Summarizing, the result in Figure 5.6 shows some specific configurations in multiparameter perturbation that lead the system to a nonrobust behavior immediately. In particular, we found that the system is very sensitive to parameter 41 and changes in this parameter must be very limited to preserve normal behavior of the system.

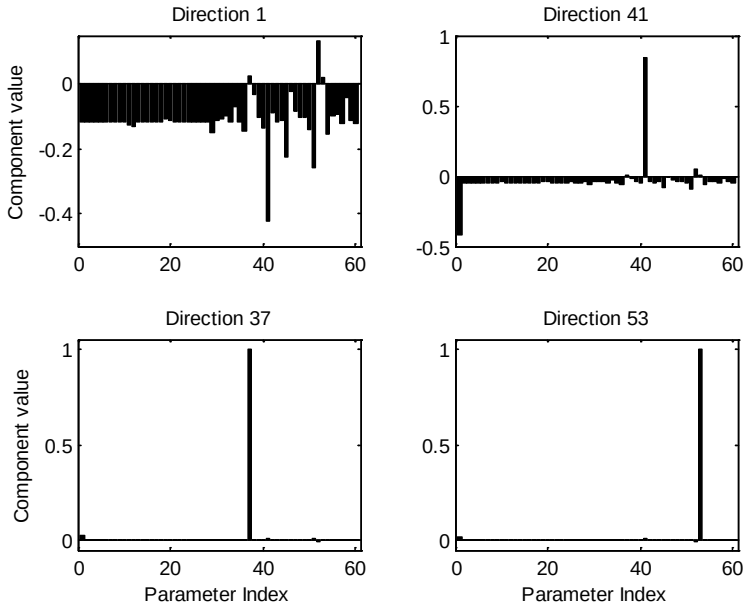

Figure 5.5 *Components of 4 perturbation vectors (selected from the in total 60 perturbation vectors). In perturbation direction 1, components 41, 51, 45, and 52 are considerably larger than the others. In direction 41, components 1 and 41 are clearly dominating. In all other directions only 1 component is dominating, as shown for directions 37 and 53. Note the scale differences between the panels.*

Table 5.1 *Five parameters for which the (combined) change in values immediately leads to an irregular cycle.*

Parameter	*Description*	*Perturbed in direction 1 or direction 41*
1	Synthesis rate constant of GnRH in the hypothalamus	1
41	Maximum increase of CL stimulated by itself	1, 41
45	Proportionality factor of CL in P4 increase	41
51	Maximum increase of OTR stimulated by P4	41
52	Threshold of P4 to stimulate OTR increase	41

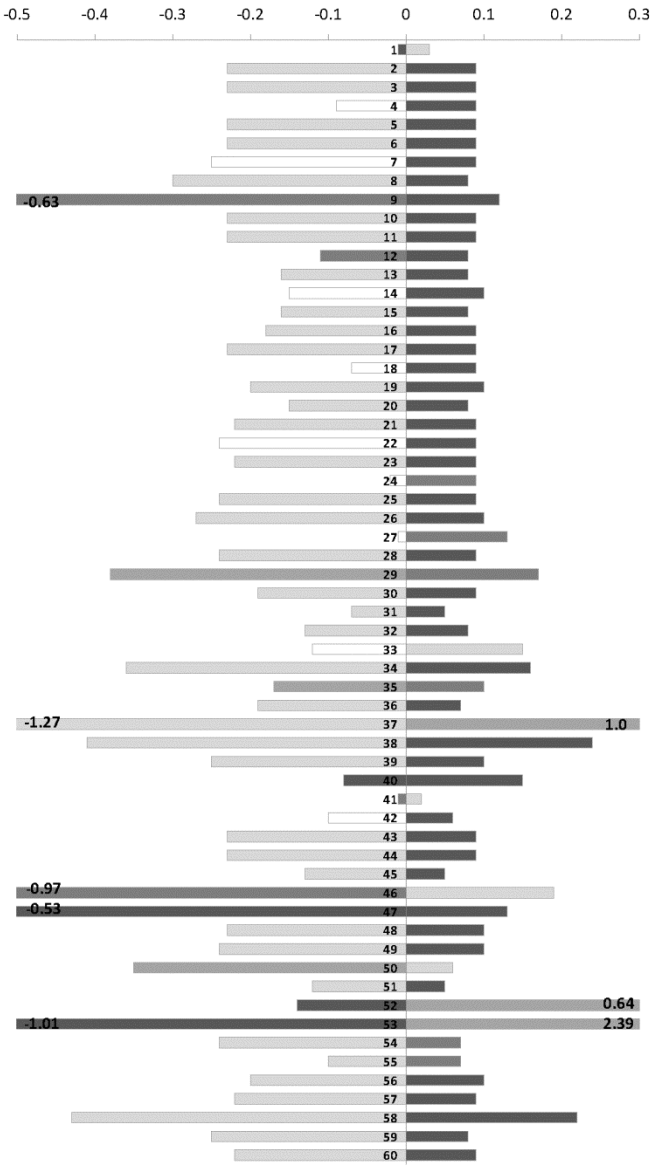

Figure 5.6 *For each of the 60 perturbation directions it was checked how far the direction could be followed before the boundary of the robustness region is reached. All directions are followed in both positive and negative direction, starting at the nominal set of parameter values. The lengths of the bars indicate how far a perturbation direction can be followed in negative and positive directions such that a normal cycle is found. Perturbation was stopped when one of the parameters passed zero (□), when P4 levels are above 2.00 (■), when delayed ovulation (■) or delayed luteolysis (□) was detected, or when delayed ovulation as well as delayed luteolysis was detected in a series of successive cycles (■).*

5.3.2 Progesterone patterns associated with delayed ovulation and delayed luteolysis

For 105 out of the 120 perturbation directions, the system became nonrobust because of the occurrence of delayed luteolysis or delayed ovulation, or both (Figure 5.6). When the luteal phase was longer than 19 days (i.e., when delayed luteolysis was detected), cycles contained 4 waves of follicle growth (indicated by E2 levels; Figure 5.7). The luteal phase length increased further when the perturbation was continued, resulting in an increasing number of waves per cycle. When the interluteal interval was longer than 12 days, and delayed ovulation was detected, the LH surge was delayed (Figure 5.8). A pattern of successive cycles with delayed ovulation was never observed. Although parameter configurations resulting in delayed luteolysis could show normal oscillatory behavior, though with more than 3 waves of follicle development per cycle, the observed parameter configurations resulting in delayed ovulation always had irregular P4 patterns (as in Figure 5.8).

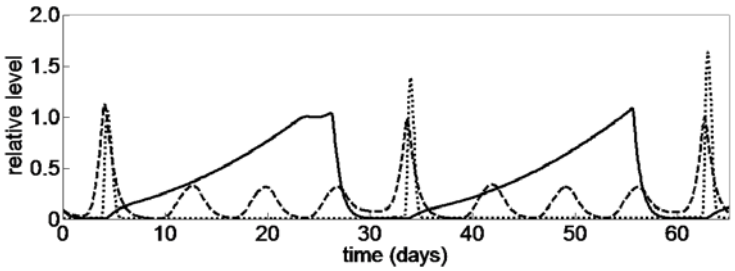

Figure 5.7 *Relative levels of P4 (solid line), E2 (dashed line), and LH (dotted line) at perturbation direction $k_0 + 0.12 \times v_9$, where k_0 = nominal parameter set and v_9 = perturbation direction 9, representing the main pattern of model output curves associated with delayed luteolysis.*

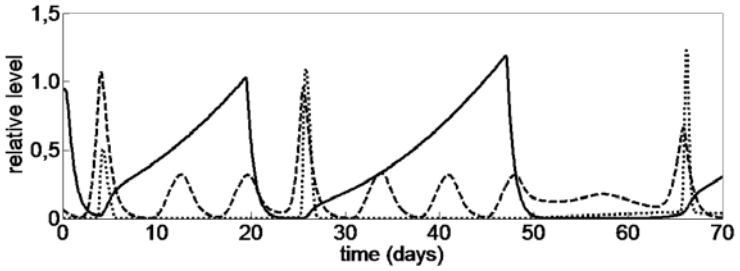

Figure 5.8 *Relative levels of P4 (solid line), E2 (dashed line), and LH (dotted line) at perturbation direction $k_0 + 0.10 \times v_{39}$, where k_0 = nominal parameter set and v_{39} = perturbation direction 39, representing the main pattern of model output curves associated with delayed ovulation.*

For 36 out of the 60 directions, it was observed that when perturbation in a negative direction was stopped because of delayed ovulation, perturbation in a positive direction stopped because within a series of successive cycles, some cycles with delayed ovulation and some with delayed luteolysis occurred (Figure 5.6). A typical series of cycles with both delayed ovulation and delayed luteolysis is shown in Figure 5.8, where the first cycle is normal, the next shows delayed luteolysis, and the third shows delayed ovulation. It also happened that one or more normal cycles occurred in between a cycle with delayed luteolysis and a cycle with delayed ovulation. Interestingly, for these 36 directions, continued perturbation in negative direction resulted again in a series of estrous cycles with normal oscillatory behavior, though with 2 waves of follicular development instead of 3, whereas continued perturbation in a positive direction resulted in a series of cycles with delayed luteolysis, containing 4 follicular waves (as in Figure 5.7). Summarizing, for these 36 directions a shift from a cycle with 2 follicular waves to a cycle with 3 follicular waves (i.e., the nominal settings) involves a transition period with irregular P4 patterns; likewise for a shift from 3 to 4 waves (i.e., delayed ovulation) per cycle (Table 5.2). As observed already in the cross-section of the robustness region in the 2-dimensional (k_{41}, k_{51}) plane (Figure 5.4), configurations with irregular hormone patterns could, thus, lie in between configurations with normal oscillatory behavior.

Table 5.2 *Types of P4 patterns observed for perturbation of 36 out of the 60 directions.*

Parameter configuration	*P4 pattern and follicular wave pattern*
Continued perturbation in negative direction	Normal cycles without variation[1] with two follicular waves
Perturbation in negative direction	Variable cycles with delayed ovulation
Nominal set	Normal cycles without variation with three follicular waves
Perturbation in positive direction	Variable cycles with delayed ovulation and delayed luteolysis[2]
Continued perturbation in positive direction	Successive cycles of delayed luteolysis without variation

[1] *P4 patterns are similar in each successive cycle.*

[2] *Within a series, some cycles have delayed ovulation and some have delayed luteolysis.*

5.4 Discussion

5.4.1 Method of robustness region estimation

In the present paper, the robustness region in parameter space of a mathematical model of the bovine estrous cycle was estimated. Within the robustness region a normal estrous cycle is predicted, and on the boundaries the dynamic behavior of

the cycle undergoes a transition, indicating that the system is no longer robust when the parameters are perturbed further. A first approximation for the robustness region in the 60-dimensional parameter space was found by perturbing the nominal parameters set along 60 perpendicular directions. Because each perturbation direction can be followed in positive and negative direction, in this way 120 points on the boundary of the robustness region were obtained. From the checked output curves for E2 and LH, it appeared that evaluation of the system based on P4 profiles only is a reliable method to detect for which parameter region a normal estrous cycle is simulated, because these curves were in accordance with the P4 profile. Parameter perturbation resulted in delayed ovulation or delayed luteolysis for as many as 105 out of the 120 points on the boundary of the robustness region. In dairy cows, delayed ovulation and delayed luteolysis are associated with the formation of cystic follicles and persistent CL, respectively (Darwash et al., 1998), which is indeed a common cause of reproductive failure. The fact that other cycle abnormalities were not observed might reflect the chosen criteria for a normal cycle used in this study.

5.4.2 Course of progesterone patterns along perturbation directions

In line with experimental data described in the literature, simulated cycles with delayed ovulation or delayed luteolysis continued to have follicular waves (Amer and Mahdi, 2008). When the model predicted a pattern of successive cycles with delayed luteolysis, the estrous cycle showed regular oscillatory behavior but contained 4 waves of follicular development due to a prolonged luteal phase. Probably this should not be considered as a pathological condition, because estrous cycles with 4 follicular waves are occasionally observed in healthy fertile cows. However, it is clear that normal cyclicity is impaired when within a series of successive cycles some cycles with delayed ovulation and some with delayed luteolysis occurred. The course of P4 profiles described in Table 5.2 shows that parameter configurations of delayed ovulation and delayed luteolysis can lie in between configurations with normal oscillatory behavior for different numbers of follicular waves. Although it is difficult to define if this is a likely physiological configuration in cows, this could represent a situation of resumption of normal cyclicity (e.g., after parturition). The observed irregular patterns in the model where delayed ovulation as well as delayed luteolysis occur suggest that delayed ovulation and delayed luteolysis in reality could have the same cause and are characterized by irregular hormone patterns. These irregular hormone patterns might explain why it is difficult in practice to give a clear pathology of cystic ovaries and why outcomes of treatments are variable (Garverick, 1997).

5.4.3 Factors that may lead to delayed ovulation and delayed luteolysis

Perturbation vectors responsible for significant qualitative transitions in the behavior of the system, causing nonrobustness, mirror the combined effects of several perturbed parameters. However, perturbation along 2 specific vectors was found to cause nonrobustness of the system immediately: for directions 1 and 41, a very small perturbation in a negative direction resulted in delayed luteolysis (and for direction 1 in delayed ovulation as well), and a very small perturbation in a positive direction resulted in delayed ovulation. In direction 1, parameters 41 (maximum increase of CL stimulated by itself), 45 (proportionality factor of CL in P4 increase), 51 (maximum increase of OTR stimulated by P4), and 52 (threshold of P4 to stimulate OTR increase) are more perturbed than the others. In direction 41, parameters 1 (synthesis rate constant of GnRH in the hypothalamus) and 41 are most perturbed. This suggests that these parameters strongly determine the occurrence of P4 patterns that are associated with delayed ovulation and delayed luteolysis in the model.

Synthesis rate constant of GnRH in the hypothalamus (parameter 1) affects the amount of hypothalamic GnRH that is produced. A perturbation of GnRH synthesis could inhibit the surge of GnRH and subsequently of LH, thereby blocking ovulation and inducing the formation of a cystic follicle. Delayed ovulation, which indicates cystic follicle formation, occurred in the model in absence of an LH surge. This phenomenon has also been observed in practice and may be the result of hypothalamic insensitivity to E2 caused by inadequate exposure to P4 (Gumen and Wiltbank, 2002). Hypothalamic content of GnRH is lower in cows with cysts (Garverick, 1997). Ovulation of a cystic follicle after GnRH treatment usually does not occur (Garverick, 1997), but in the model we could not check whether the GnRH/LH surge following delayed ovulation causes ovulation or only luteinization of the follicle, because the event of ovulation is not separately included in the model.

Maximum increase of CL stimulated by CL itself (parameter 41) in the model captures physiological processes such as local effects of OT, PGF2α, and noradrenaline on CL development and maintenance (Skarzynski et al., 2008). From a biological perspective, this could translate to the fact that a proper functioning of the CL (i.e., sufficient P4 production) is crucial for normal cyclicity. Maximum increase of OTR stimulated by P4 (parameter 51) and threshold of P4 to stimulate OTR increase (parameter 52) affect the time point of luteolysis. It may seem obvious and not surprising that parameters regulating the time point of luteolysis are involved in the occurrence of P4 profiles associated with delayed ovulation and delayed luteolysis. However, it is likely that in many other perturbation directions

the change in P4 profiles is the indirect effect of many small changes in other parameters. Moreover, all model components are connected and more or less affecting each other, which makes it hard to predict beforehand which specific mechanisms are involved in the development of cystic ovaries. Experiments to verify model predictions would require measurements during successive estrous cycles because it is unpredictable when development of cystic ovaries occurs. Further, such experiments should go beyond blood sampling because this would only identify the occurrence and not the underlying causes of cystic ovaries, which according to the simulation results are more likely at a cellular level.

5.5 Conclusions

The method of robustness region estimation used in the present study is an effective tool to find parameter configurations that lead to specific endocrine profiles in a model of the bovine estrous cycle, such as P4 profiles associated with delayed ovulation and delayed luteolysis. Thereby, the model can help to generate hypotheses regarding the mechanisms and predisposing factors involved in the development of cystic ovaries. The simulation results presented here support that mechanisms regulating CL functioning, luteolytic signals, and GnRH synthesis are likely to be involved in the development of cystic ovaries. In the multidimensional parameter space, areas exist in which the parameter configurations resulted in normal cycles. These areas may be separated by areas in which irregular cycle patterns occurred (see Figure 5.4 for an example). These irregular patterns thus mark the transition from one stable (normal) situation to another. Interestingly, within a series, some cycles had delayed ovulation and some had delayed luteolysis in these patterns. The onset of cyst development in cows is currently unpredictable, but the model predictions presented in this study indicate some candidate mechanisms to consider for further investigation.

Acknowledgements

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6

Parameterization of a mathematical model of the bovine estrous cycle for cows with different estrous cycle characteristics

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Abstract

We used a recently developed mathematical model of the bovine estrous cycle. The model describes the cyclical fluctuations of hormones, follicles and corpora lutea with a set of linked differential equations. The initial model parameterization resulted in estrous cycles with 3 waves of follicular growth. In earlier studies we changed parameters values and found alternative parameterizations for cycles with two follicular waves and for cystic ovaries respectively. In the current study we have applied the model to fit empirical datasets of 31 individual cows, with the objective to identify mechanisms that explain individual differences in cycle characteristics. We did this by identifying parameter configurations for the mathematical model that describe the *in vivo* measurements on follicle and CL sizes, P4, E2, FSH, and LH concentrations in plasma for each cow, using a least squares optimization procedure. The model appears to be able to accommodate normal variation in estrous cycle characteristics of cows. With the parameter sets estimated for the individual cows, the model showed correct or at least improved predictions for the number of follicular waves for 21 out of 30 cows of which the number of follicular waves was known. Estimation of a number of parameters confirmed results of previous model simulations indicating that parameters involved in luteolytic signaling are very important for regulation of general estrous cycle characteristics and are likely responsible for differences in estrous cycle characteristics between cows.

Key words: mathematical model, bovine estrous cycle, parameter identification

6.1 Introduction

Recently we developed a deterministic mathematical model that describes the dynamics of the bovine estrous cycle as a set of linked differential equations. The model generates cyclical fluctuations of hormone concentrations, follicles and corpora lutea in estrous cycles of approximately 21 days for cows with a normal estrous cycle. The parameters in the model represent kinetic properties of the system with regard to synthesis, release and clearance of hormones, and growth and regression of follicles and corpora lutea (Boer et al, 2011b). The purpose of this model is to test and generate hypotheses regarding regulation of the bovine estrous cycle and dairy cow fertility. Appropriate parameterization is of great importance in the development, validation and use of the model. As a first step in model validation, it was demonstrated that it is possible to find parameter values that can simulate a normal 'average' cow (Boer et al., 2011b). Another test of the model was that it is possible to find parameter values to simulate disruptions of normal cyclicity like cystic ovaries (Boer et al., 2012). In the current study we investigated whether the model is able to accommodate normal variation in estrous cycle characteristics of individual cows.

For a mathematical model of a complex dynamic system, a main difficulty is to estimate parameter values for the unknown parameters that result in simulation output curves that agree with the experimental data. Most parameter values (i.e., the level of biological responses) in the model are yet unknown because no *in vivo* measurements are available. In our model of the complex system of the bovine estrous cycle this leads to a large number of unknown parameters, and estimating all parameters simultaneously is impossible because parameters are dependent on each other. Boer et al. (2011b) used a model decomposition approach to obtain the initial model parameterization, i.e., 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 and subsequently replaced the input curves, until finally a closed network was obtained. Estimation of the initial parameter values was thus based on average hormone profiles obtained from several data sources published in literature (Boer et al., 2011b).

It is an important step in model validation to estimate parameter values from an independent experimental dataset that was not used for initial model parameterization. In previous studies it was investigated whether it was possible, starting with the initial parameter values, to induce parameter perturbations that

matched with certain estrous cycle characteristics like the number of follicular waves (Boer et al., 2011a) or cystic ovaries (Boer et al., 2012). In the current study, we did not take the model, but empirical estrous cycle characteristics data as a starting point to generate specific parameterizations that would yield output curves that match these cycle characteristics. Thirty-one cows with synchronized estrous cycles provided the *in vivo* measurements on follicle and CL sizes, P4, E2, FSH, and LH concentrations in plasma. We estimated a number of parameter values and investigated which differences in parameter values were found for cows that differ in estrous cycle characteristics like follicle wave number and peripheral blood P4 levels. This generated hypotheses about which mechanisms lead to different estrous cycle characteristics, and how this could affect reproductive performance. The main objective of this study is to investigate these underlying mechanisms resulting in differences between estrous cycles. To achieve this, the model has to be able to represent individual cow data accurately without extreme, probably non-physiological parameter values. In particular, this study assessed the capability of the model to simulate 'real' data by finding specific model parameterizations for individual cows.

6.2 Material and methods

This section briefly describes the mathematical model of the bovine estrous cycle and the dataset that was used to validate parameter values of this model. In particular the procedure for parameter identification is explained. Model parameter values were fitted such that the simulated curves match the given *in vivo* data using a least squares optimization procedure.

6.2.1 Mathematical model

We used the deterministic mathematical model of the bovine estrous cycle originally described by Boer et al. (2011a) and Boer et al. (2011b) with the modifications introduced more recently by Stötzel et al. (2012). Briefly, the model describes the dynamics of the bovine estrous cycle as a set of 15 linked differential equations (Figure 6.4) with 60 parameters, including the process of follicle and CL development and the relations with key hormones that interact to control these processes. In the model, follicles are not modeled separately, and 'Follicle' represents the capacity of follicles present at any moment to produce E2 and Inh, rather than follicular size. Likewise, 'CL' represents the capacity of the CL to produce P4. The initial parameterization (Boer et al., 2011b) of this model results in estrous cycles with three waves of follicular development. A complete overview of all equations and initial parameter values can be found in Boer et al. (2012) and

online (https://github.com/CSB-at-ZIB/BioPARKIN/downloads) in *SBML* (Systems Biology Markup Language) code.

6.2.2 Experimental data

6.2.2.1 Dataset description. Estrous cycle related physiological measures of cows with similar proportions of Holstein genetics, similar genetic merit for milk production traits, but with good (Fert+) or poor (Fert-) genetic merit for fertility traits were used. Good genetic merit for fertility involves a negative estimated breeding value (EBV) for calving interval and a positive EBV for survival, according to the Economic Breeding Index (EBI) in Ireland. A detailed description of the animals and treatments was reported by Cummins et al. (2012). In summary, 19 Fert+ and 12 Fert- cows with synchronized estrous cycles underwent daily ultrasonography during the cycle that followed directly after the synchronized ovulation. 20 cows had a cycle with 2 follicular waves, 10 cows had a cycle with 3 or more follicular waves, and for one cow the number of waves was not clear. Thus, the dataset consisted of 15 Fert+ cows with 2 waves, 4 Fert+ cows with 3 or more waves, 5 Fert- cows with 2 waves, and 6 Fert- cows with 3 or more waves. Blood sampling was carried out at 8 h intervals from d 0 to d 6 of the cycle and from day 15 to ovulation, and once daily from day 7 to day 15. Daily blood samples were analyzed for P4 during the entire cycle, for E2 and FSH from day 0 to day 6 and from day 15 until ovulation, and for LH from day 15 until ovulation (Figure 6.1).

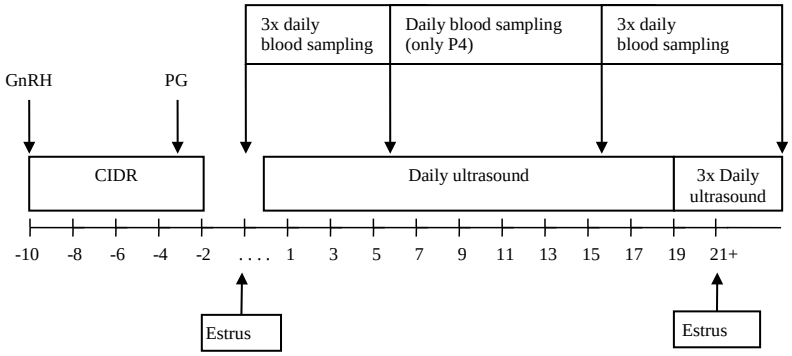

Figure 6.1 *Synchronization protocol, blood sampling and ultrasound frequency for one complete estrous cycle relative to day of ovulation (d 0). CIDR = intravaginal P4-releasing device containing 1.38 g of P4; GnRH = GnRH agonist injections contained 10 µg buserelin; PG = prostaglandin F2α analogue (adapted from Cummins et al., 2012).*

6.2.2.2 Processing of the data in the model. The software package BioPARKIN, (Dierkes et al., 2011) was used to fit the model parameter values such that the simulations match the given data. Before importing the data files for each individual cow in BioPARKIN, the empirical data for P4, E2, FSH and LH were scaled, to get a good initial estimate of the parameter values. The default model parameterization leads to dimensionless simulations on a relative scale, and therefore by default result in output curves between 0 and 1. The initial parameterization thus scaled the hormone levels to the same order of magnitude. This is, however, different from empirical data. One possibility to account for the different dimensions is to scale certain model parameter values such that the simulated curves have physiological dimensions and units. The other possibility, chosen in the current study, is to scale the experimental data to the relative scale of the default model. Therefore, the levels of P4, E2, FSH and LH of each individual cow were divided by the mean value of the peak levels of all cows for the respective hormones (Table 6.1). This resulted in peak values around 1 in line with the initial simulated curve but still representing the variation between cows. Data from one cow was excluded from the calculation of the mean peak levels because she became anoestrus and had undetectable or baseline P4 and E2 concentrations throughout the synchronized estrous cycle. Mean peak height of the LH surge of the cows was based on data from only 26 cows as for four cows the LH peak could not be recognized. These cows all belonged to the Fert- group. Since the LH surge is short, the time interval between LH measurements was probably too long to detect the exact peak of the LH surge. Therefore the peak value of LH may be underestimated. A similar scaling procedure was followed for the CL volume. Where the CL had a cavity, the cavity volume was subtracted from the total CL volume. For the follicle data, diameter of the dominant follicle of each follicular wave was taken, and these diameters were summed (Figure 6.2), as a single curve

Table 6.1 *Means of the peak hormone levels or peak size of follicle and CL of 30 cows. These means are used to scale the empirical data to be in line with the initial simulated curves on a relative scale.*

Component	Mean peak level	Range
P4	8.76 ng/ml	5.59-14.92
FSH	0.40 ng/ml	0.19-0.83
E2	4.40 pg/ml	0.75-10.68
LH[1]	4.03 ng/ml	0.74-7.90
Follicle[2]	27.8 mm	18.0-36.9
CL	11.4e3 mm^3	4.6-21.8E3

[1] *mean of 26 cows for which LH was measured*

[2] *diameters of dominant follicles added together*

for the follicle data was required because follicular function in the model is represented by a single equation. This approach assumes that the dominant follicle is the main producer of E2 and Inh (Boer et al., 2011b). To scale these follicle data, the measurements of the summed diameters per cow were divided by the mean peak value of these summed diameters. Data from one cow was excluded from calculation of the mean follicle levels, because the number of follicular waves could not be clearly recognized. Figure 6.3 shows an example of the scaled data points for an individual cow as they were imported in BioPARKIN.

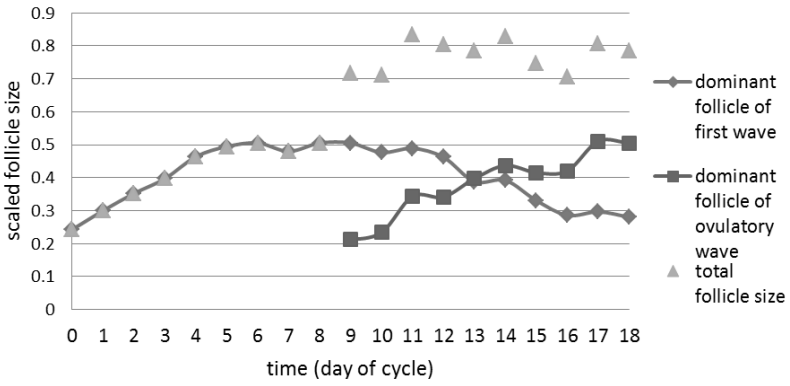

Figure 6.2 *Example of empirical data for the (dominant) follicle of an estrous cycle with two waves of follicular development imported in BioPARKIN. The scaled diameters of the two dominant follicles were summed to obtain total follicle size, and model parameter values were fitted on the resulting data points.*

6.2.3 Parameter identification

Identifying the parameter settings that lead to closest agreement between model output and data points for each cow is in essence a nonlinear least squares problem. However, solving is difficult because several combinations of parameter values may give simulations that explain the data equally good. Also, the potential range of parameter values is very large. To restrict the parameter space and thus facilitate optimization, the parameter ranges were constrained to 10-fold the initial value, also to prevent the model from estimating values that were likely to be non-physiological. The *SBML*-compatible software package 'BioPARKIN' (developed at the Zuse Institute Berlin) was used for identification of model parameter values. The algorithms underlying this program indicate the subset of parameters that can be identified from the given data. It optimizes these parameter values to obtain the least squares best fit to the data, thus minimizing the deviation between model and

data at the measurement time points. The procedure used to solve the nonlinear least squares minimization problem is described in more detail in Dierkes et al. (2011).

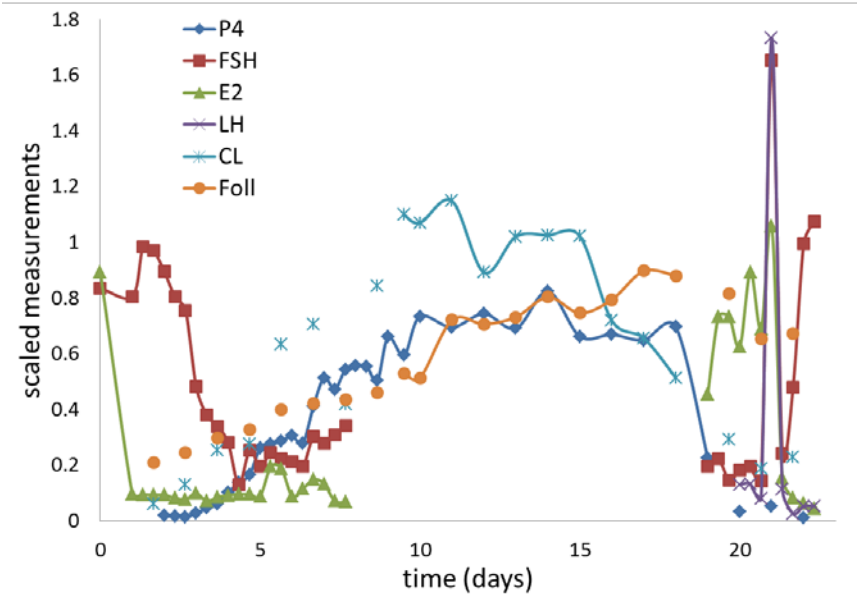

Figure 6.3 *Example of scaled measurement data of an individual cow.*

Since the least squares method analyzes the 'vertical distances' between simulated and empirical data, (i.e., not across time), time shifting was applied to the data that resulted in an optimal overlap between the initial curves and the imported data in order to obtain the best initial guess in fitting the curves. A general sensitivity analysis was performed to find out at which time point in the cycle the sensitivities of P4 to the 60 model parameters are highest. The parameters with high sensitivities and low subcondition at this time point were fitted first. The subcondition is calculated from the sensitivity matrix and is used for rank decision in the optimization algorithm. It indicates whether a parameter can be estimated from the given data or not and thus gives some information about the dependency of a parameter on other parameters (Dierkes et al., 2011). Variation of parameters with very low sensitivity has only small influence on the model solution. These parameters are therefore difficult to estimate (since the model solution often will not converge because a wide range of parameter values is possible). Taking the parameters with the highest sensitivities and the lowest subconditions gives a first subset of parameters. After fitting this first subset of

parameters for each cow, parameters were fitted that in previous studies were identified to play an important role in regulation of follicular wave pattern (Boer et al., 2011a) and of P4 profiles (Boer et al., 2012). Curves simulated by the model were visually assessed for agreement with empirical endocrine profiles and known estrous cycle characteristics. Estimated parameter values for different groups were tested for statistical significance (e.g. groups that differ in wave numbers and EBV for fertility). For the set of fitted parameters, a sensitivity analysis was performed for each cow to see whether the order of sensitivities and subconditions was changed. An overview of the iterative process of parameter fitting is shown in Figure 6.4.

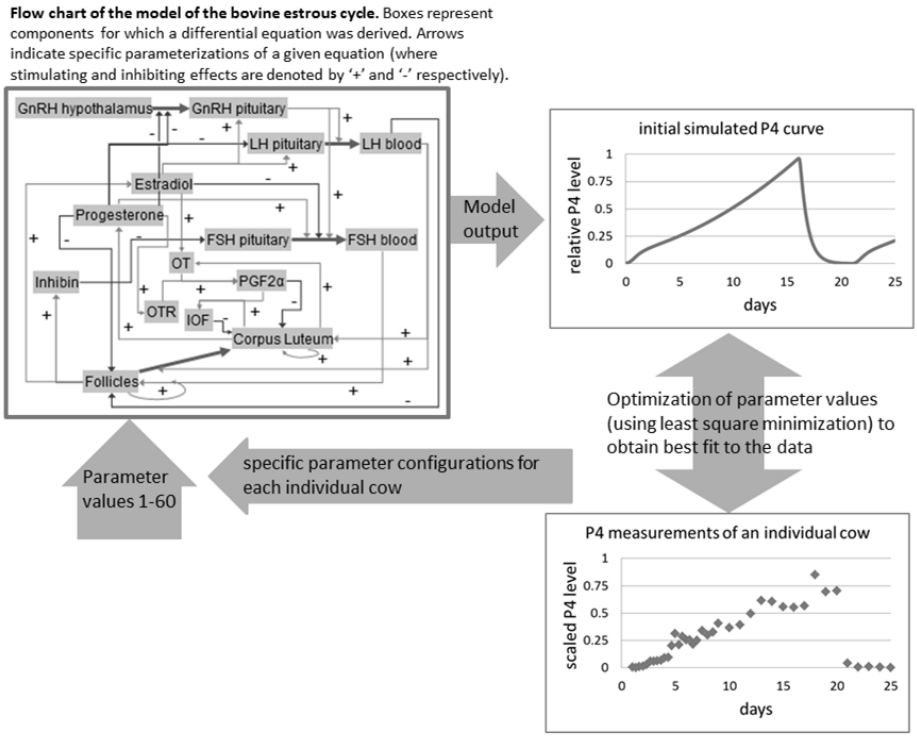

Figure 6.4 *Overview of the iterative process of parameter fitting, illustrated with P4 (progesterone) profile. Besides P4, data for LH, FSH, E2 (estradiol), follicle, and CL (corpus luteum) are simultaneously included in the least squares procedure used to fit a subset of the parameters.*

6.3 Results and discussion

6.3.1 Parameter identification

The general sensitivity analysis of the initial model parameterization showed that P4 is most sensitive to parameter changes around the time point of onset of luteolysis (i.e., when the slope of P4 is most negative) at day 16.3 (Figure 6.5). The ten parameters for which P4 was most sensitive also have the highest sensitivities for CL. Of these ten parameters, five parameters (parameter 38, 51, 44, 33, and 49) had a low subcondition. A description of these and the other fitted parameters is given in Table 6.5. Parameter 38, 51 and 44 are involved in luteolysis, parameter 33 affects follicular function, and parameter 49 is involved in Inh production. These five parameters were fitted first (Table 6.2). The estimated values for all of these five parameters varied between cows, especially parameter 38 (Figure 6.6) and parameter 33. However, the difference between the Fert+ and Fert- groups was not significant. Remarkably, the mean, minimum, and maximum estimated parameter values are almost identical for the cows with good (Fert+) and the cows with poor EBV for fertility (Fert-). However, the values vary widely within these groups (Table 6.2). This indicates that the difference in estimated value for these parameters is likely caused by factors other than EBV for fertility.

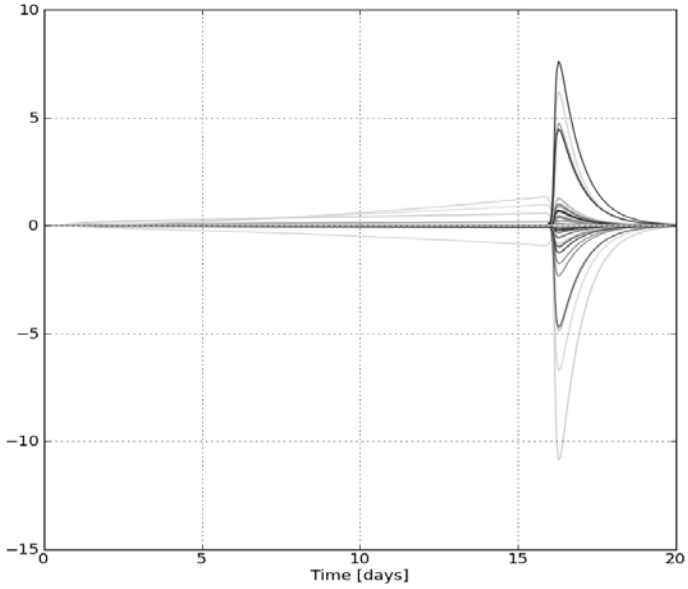

Figure 6.5 *Sensitivities of P4 to the 60 model parameters as a function of time of cycle. Each line represents the absolute sensitivity of P4 to one parameter. Sensitivities are highest immediately after the onset of luteolysis on day 16.3.*

Table 6.2 *Overview of estimated parameter values starting from the initial model parameterization for the five parameters (with the other parameters fixed at their initial value) with low subcondition and high sensitivity for P4.*

		All cows (n=31)				Fert+ cows (n=19)				Fert- cows (n=12)			
Par	Initial	No.	Mean	Min	Max	No.	Mean	Min	Max	No.	Mean	Min	Max
38	1.09	20	1.23	0.16	2.22	11	1.18	0.52	2.22	9	1.29	0.16	2.05
51	3.58	27	3.62	3.00	4.45	16	3.64	3.00	4.45	11	3.60	3.00	4.33
44	1.32	25	1.14	0.51	5.33	14	1.16	0.51	5.33	11	1.12	0.53	1.88
33	0.13	28	0.26	0.03	1.18	18	0.22	0.12	0.51	10	0.32	0.03	1.18
49	1.41	30	1.38	1.13	1.56	19	1.38	1.13	1.52	11	1.39	1.17	1.56

No.: number of cows for which the respective parameter could be estimated

From a biological point of view, the sensitive parameters with high variation in estimated value are likely the most interesting parameters. However, it could also be that a parameter with a small variation in estimated values (but a very high sensitivity) is interesting (e.g. parameter 49), as this would indicate that only a small variation is sufficient to obtain a good fit. Another explanation of the differences in variation of estimated values could be that the model 'chooses' to vary a specific parameter (e.g. parameter 33) more than other parameters to fit the curves. The variation in estimated values is larger in the group of Fert- cows compared to the Fert+ cows. The deviating values in some Fert- cows might indicated that something is 'wrong' in these cows. When estimated parameter values for different cows are closer together, as is the case in the Fert+ group, this could mean that the optimal values have been found for this group of cows. These

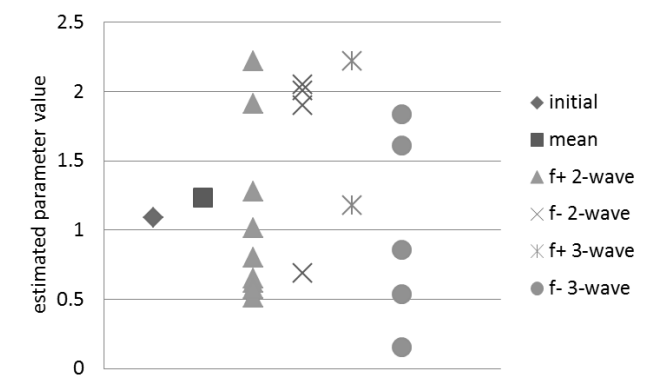

Figure 6.6 *Illustration of estimated value per cow for parameter 38 (threshold of oxytocin to stimulate PGF2α increase). Initial: initial parameter value; Mean: mean of estimated (fitted) values; f+: good EBV for fertility; f-: poor EBV for fertility; 2-wave: two waves of follicular development per cycle; 3-wave: three or more follicular waves.*

values might be fixed for the next iterations. When estimated parameter values highly differ this could mean that they explain differences in estrous cycle characteristics between cows. The highest value for parameter 33 was an outlier and belongs to the one cow in the dataset with five follicular waves. This is not surprising, since a high value of parameter 33 (threshold of P4 to stimulate decrease of follicular function) will result in a lower inhibitory effect of P4 on follicular growth. The effect of P4 on follicular growth (partly represented by parameter 33) , together with the effect of Inh (partly represented by parameter 49), affect the time point at which the dominant follicle of a wave goes into regression. The highest value for parameter 44 (Threshold of IOF to stimulate CL decrease) was an outlier belonging to a cow with high P4 levels for a number of days after the relatively large CL started to regress. It is therefore not surprising that a deviating value was found for a parameter involved in luteolysis. These outliers are a validation of the model, because they confirm model behavior in deviating physiological settings.

6.3.2 Follicular wave pattern

Using the estimated values for the individual cows for the five parameters presented in Table 6.2 as fixed values, three additional parameters were fitted that in a previous study with the model (Boer et al., 2011a) were found to affect the number of follicular waves via changes in follicular growth rate (parameters 29, 31, and 32, Table 6.3). Most variation in estimated value was found for parameter 31, but the difference between cows that had a cycle with 2 waves or cows that had a cycle with 3 or more waves of follicular development was not statistically significant.

Table 6.3 *Means and ranges of estimated values of three parameters which are reported to affect follicular wave pattern. For the five parameters presented in Table 6.2 the estimated parameter values for the individual cows were used in this iteration within the model.*

		All cows (24/31)			2 waves (16/20)			3 or more waves (8/10)		
Par	Initial	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
29	0.56	0.47	0.20	0.99	0.49	0.20	0.99	0.44	0.26	0.77
31	0.22	0.16	0.01	0.85	0.13	0.03	0.37	0.21	0.01	0.85
32	1.1	1.17	0.59	2.45	1.23	0.60	2.45	1.05	0.59	1.72

Because we wanted to optimize the parameterization of follicular function, one research question was how well the number of follicular waves was predicted. The previous study with the model on follicular wave patterns showed that the number of waves is not only affected by follicle growth rate but also by time point

of luteolysis (Boer et al., 2011a). The modeling of luteolysis has changed in the model used here, but the wave pattern effects identified by Boer et al. (2011a) would in the present model translate to changes in par 37, 51, and 52. Since par 51 was already fitted, a next fitting procedure was performed for par 37 (Threshold of OTR to stimulate PGF2α increase) and 52 (Threshold of P4 to stimulate OTR increase) (Table 6.4), in which for the parameters presented in Tables 6.2 and 6.3 the estimated parameter values for the individual cows were used. There was a numeric (but not significant) difference for the estimated value of par 52 (Threshold of P4 to stimulate OTR increase) between cows with 2 waves and cows with 3 or more waves. Because a previous study with the model indicated that parameter 41 has a large effect on luteal phase length (Boer et al., 2012), this parameter was also fitted (after fitting par 37 and 52). However, estimated values for parameter 41 were all very close to the initial value of 0.04.

Table 6.4 *Means and ranges of estimated parameter values for parameters 37 and 52. For the parameters presented in Tables 6.2 and 6.3, the estimated parameter values for the individual cows were used in this iteration within the model.*

		All cows (18/31)			2 waves (9/20)			3 or more waves (8/10)		
Par	Initial	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
37	1.43	1.56	1.23	2.30	1.63	1.23	2.30	1.51	1.27	1.83
52	0.77	0.72	0.25	0.96	0.67	0.25	0.96	0.77	0.68	0.88

After thus fitting the total of 11 parameters, the number of follicular waves was predicted correctly for 15 cows, and the model simulations for follicular function visually matched better to the data with the 'new' parameterization than with the initial parameterization for a further seven cows. Thus, in total for 22 out of 30 cows for which the number of follicular waves was known the model gave correct or at least improved predictions for the number of follicular waves. Due to individual parameterization of these 11 parameters, the simulated wave pattern was changed compared with the initial simulated curves for 21 cows. For 18 of these 21 cows, the change in wave pattern visually matched better to the data than the initial simulated curves. Of the four cows that had four waves, the model predicted a 3-wave cycle for three cows, and an irregular pattern was predicted for the fourth cow. For the five cows that had a three-wave cycle, the 'new' parameter settings still predicted a three-wave cycle. For the other two of these five cows, the second follicular wave in the output curves did not completely regress (Figure 6.7). A similar 'intermediate' pattern between 2-wave and 3-wave cycles was also predicted for four of the 20 cows with a 2-wave cycle. For 13 of the 20 cows with a 2-wave cycle, a 2-wave cycle was predicted for the simulated cycle in which the

empirical data was overlaid (Figure 6.8) or for the subsequent simulated cycle (Figure 6.9).

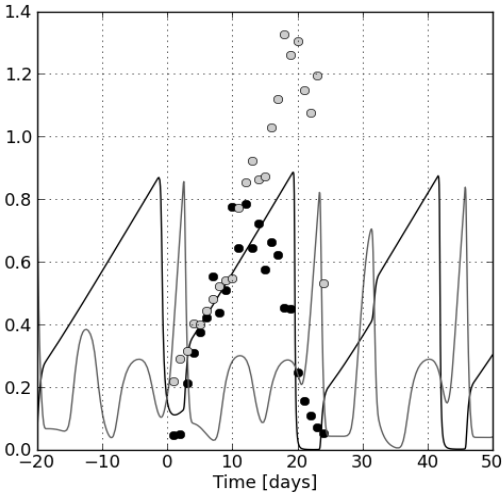

Figure 6.7 *Example of model predictions and data points for CL (black line, black bullets) and follicle (grey line, grey bullets) for an individual cow after estimating the eleven parameters mentioned in Table 6.5. The model was simulated for three to four cycles and the empirical data were overlaid over the second simulated cycle. The second follicular wave of this simulated cycle does not completely regress. No dimension is given at the y-axis because the equations are expressed on a relative scale.*

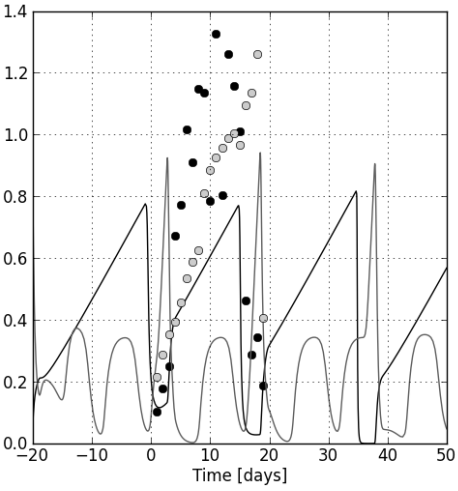

Figure 6.8 *Example of model predictions and data points for CL (black line, black bullets) and follicle (grey line, grey bullets) after estimating the parameters mentioned in Table 6.5. Parameter values were fitted to one of the cows with two follicular waves, resulting in a 2-wave simulation for the cycle in which the data points were overlaid. No dimension is given at the y-axis because the equations are expressed on a relative scale.*

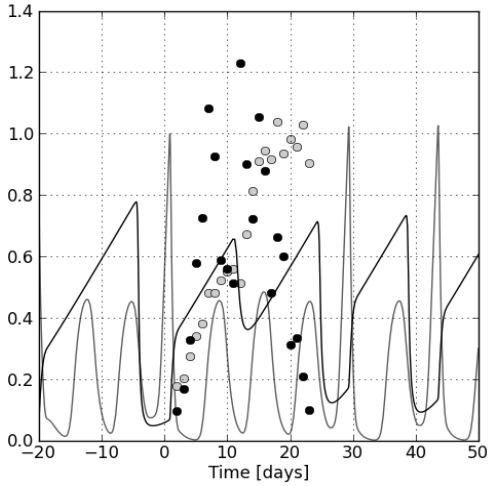

Figure 6.9 *Example of model predictions and data points for CL (black line, black bullets) and follicle (grey line, grey bullets) after estimating the parameters mentioned in Table 6.5. Parameter values were fitted to a cow with two follicular waves, resulting in a 2-wave simulation for the cycle following the cycle in which the data points were overlaid. No dimension is given at the y-axis because the equations are expressed on a relative scale.*

To see whether the parameter fitting had an effect on the sensitivities of P4 to the parameter values, after fitting the 11 parameters listed in Table 6.5 a sensitivity analysis was performed with the new parameter set for each cow. In this second sensitivity analysis, parameter 41 (which was not one of the ten most sensitive in the initial parameterization), now occurred for 12 cows in the list of ten most sensitive parameters. For 11 of these 12 cows a clear change in the number of follicular waves was observed after fitting the 11 parameters mentioned in Table 6.5. The estimated value of parameter 41 (the maximum increase of CL stimulated by itself), however, shows very little variation between cows. Furthermore, this second sensitivity analysis showed a low subcondition for five other parameters (parameter 18, 23, 55, 58, and 60) for a number of cows. Although P4 was not very sensitive to these parameters, it is possible that other model components are, and therefore it could be interesting for future work to continue the fitting procedure with these parameters.

Table 6.5 *Model parameters of which the value was estimated for each individual cow.*

No.	Symbol	Description
38	$T_{OT}^{PGF2\alpha}$	Threshold of OT to stimulate PGF2α increase
51	m_{P4}^{OTR}	Maximum increase of OTR stimulated by P4
44	T_{IOF}^{CL}	Threshold of IOF to stimulate CL decrease
33	T_{P4}^{Foll}	Threshold of P4 to stimulate decrease of follicular function
49	c_{Foll}^{Inh}	Proportionality factor of follicular function in Inh increase
29	m_{FSH}^{Foll}	Maximum increase of follicular function stimulated by FSH
31	T_{Foll}^{FSH}	Threshold of follicular function to downscale FSH threshold
32	m_{P4}^{Foll}	Maximum decrease of follicular function stimulated by P4
37	$T_{OTR}^{PGF2\alpha}$	Threshold of OTR to stimulate PGF2α increase
52	T_{P4}^{OTR}	Threshold of P4 to stimulate OTR increase
41	m_{CL}^{CL}	Maximum increase of CL stimulated by itself

The first sensitivity analysis calculated for which parameters P4 was most sensitive. To explore the effect of the chosen conditions (e.g. time point of sensitivity analysis) on parameter estimation, also the sensitivities of each parameter of the initial parameter set to follicular function was calculated. Then, starting with the initial parameter set, the parameters with high sensitivity and low subcondition (now parameters 49, 38, 24, and 14) were fitted. Parameters 49 and 38 had a high sensitivity and a low subcondition based on the sensitivity analysis for P4 as well as for follicular function. Although the estimated parameter values did show quite a lot of variation, this did not result in an altered wave pattern or altered peak values for follicular size. The estimated value for parameter 49, however, was significantly lower for cows with three or more waves of follicular development per cycle compared to cows with two waves (Figure 6.10, proportionality factor of follicular function in inhibin increase), despite the fact that there is considerable overlap of the distributions in the 2-wave and 3-wave cows. As another test of the effect of fitting parameters in another order on the estimated values, parameters 29, 31, and 32 were fitted before the five parameters presented in Table 6.2 were estimated (i.e. starting from the initial parameterization). This resulted in almost similar parameter values, but poorer performance regarding the predicted number of follicular waves.

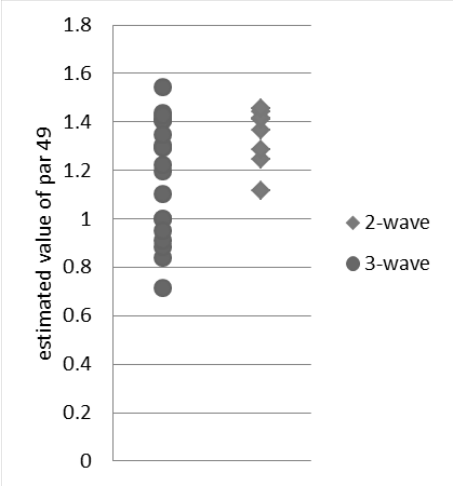

Figure 6.10 *Estimated value for parameter 49 for cows with two waves (2-wave) and cows with three or more waves (3-wave) of follicular development per cycle.*

6.4 General discussion

In the current study, a mechanistic mathematical model of the bovine estrous cycle was parameterized for an independent dataset comprising measurements of 31 individual cows. Previous validation steps were 1) creating a parameter configuration that simulates an 'average' normal cycling cow (Boer et al., 2011a) and 2) creating parameter configurations that can simulate various physiological settings by changing parameter values within different approaches (Boer et al., 2011b; Boer et al., 2012). In the current study it was investigated whether the model is able to find parameter configurations for 31 individual cows without taking extreme, probably non-physiological values. The parameter fitting delivers information on biological mechanisms underlying phenotypic variation (e.g. fertility, number of waves, P4 patterns) between cows. The fitted curves were mainly evaluated on follicular function (i.e. the number of follicular waves) and P4 profiles, because data of follicular size and P4 levels was available for the whole cycle.

6.4.1 Approach of parameter estimation

Two possible measures to assess whether the model is capable to simulate real data are 1) the estimated parameter values (are these within a range of predefined 'normal' values) and 2) the predicted endocrine profiles (do these match in vivo measurements). These two measures of estimated values and predicted profiles together are helpful to give a biological interpretation to the model predictions,

and to identify which mechanisms induce a shift in model behavior and thus regulate estrous cycle characteristics. In this study, parameters with a high sensitivity and with a low subcondition were fitted first. The resulting parameter values depend on the chosen conditions, since subconditions and sensitivities depend on the actual parameter set and are calculated only at the specified time points for the sensitivity analysis. The current study indicates that calculating sensitivities for another component (Foll instead of P4) does not result in highly different parameter values after the following optimization procedure. However, a change in component or time point of sensitivity analysis could give different sensitivities and subconditions. Therefore other parameters would be chosen to be fitted first and the order in which they will be estimated will differ. The use of a similar approach for each cow in this study (i.e. fitting the same parameters in the same order), however, allowed comparison of the results with each other. First results of parameter fitting in another order (for parameter 21, 31, and 32) did not result in major differences in the estimated values.

Estimation of single parameters (keeping all other values fixed) is likely to be successful when they have a low subcondition (i.e., they are less dependent on others within the current parameter setting). Although the first five parameters were fitted one by one (and not together), they show a large variation between cows in estimated value. In contrast, fitting of par 41 only, which has a higher subcondition, gave a very narrow range of estimated values. Fitting of parameter 41 does not give a good representation of the variation in the endocrine profiles (P4 in particular) between individuals. Therefore, we need to find other parameters that are able to predict variation in endocrine profiles between individuals. The challenge is to find appropriate combinations of parameters, but it is difficult to fit multiple parameters together as the algorithm often does not converge because of the large number of possible outcomes. The sensitivity analysis gives an order of subconditions and thus a hint of parameter combinations that are promising to be successfully fitted together. However, subconditions change as parameter values vary throughout the optimization procedure. As *a priori* information the subconditions thus has to be taken with caution. To improve this issue of finding proper parameter combinations, it is also helpful to take into account known biological mechanisms when deciding on which (combinations of) parameters to fit. Manually changing parameter values and assessing its effect on the predicted endocrine profiles can support this decision. Whether or not parameters can be estimated together may provide insight in dependencies between model mechanisms, and thus in potential interactions in the biological system of bovine estrous cycle regulation.

As already mentioned in the Introduction, it is difficult to determine correct parameter values for the model parameters from *in vivo* measurements. Moreover, collectively fitting them to other experimental data (like in this study by optimizing the agreement between the model simulation and the available data) often leaves large parameter uncertainties. The collective behavior, e.g. the resulting P4 output *in vivo*, is much easier to measure than the values of individual 'parameters' (i.e. the regulatory factors), partly since the parameters are estimated from time-course measurements of hormone concentrations and follicle and CL size. Note that the purpose of this study was not to determine the exact values of biological parameters, but to indicate which parameters could explain differences between phenotypes. The model can be used to simulate data that was not used for the original parameter estimation. This means that the model is useful for interpretation of experiments. Parameter values that optimally fit the data are not necessarily unique. Therefore, conclusions to their optimality from a biological point of view need to be drawn with caution. Some parameters can compensate or depend on other parameters and thereby cause some 'arbitrariness' in their absolute value. This relates to the problem of identifiability since different (combinations of) parameter values may be equally consistent with the data (Slezak et al., 2010).

6.4.2 Parameter settings that affect progesterone levels

Within the 31 cows used in this study, the Fert+ cows tended to have fewer follicular waves and had significantly higher peripheral blood P4 levels than Fert- cows (Cummins et al., 2012). Therefore we expected a difference between Fert+ and Fert- cows with respect to parameters involved in follicle growth rate and time point of luteolysis (mechanisms that were found to affect follicle wave pattern in Boer et al., 2011a). A reason that differences in these parameters were found neither between the Fert+ and Fert- cows nor between the 2-wave and 3-wave cows could be that a change in output curves is induced by small changes in several parameters together or that different configurations can lead to the same phenotypic output. The effects of changing specific parameters one at a time on the behavior of the model of the bovine estrous cycle was analyzed in Boer et al. (2011a). However, in the reality of biological systems it is more likely that a perturbation is the effect of simultaneous changes in multiple physiological parameters, as can be simulated in the model by multi-parameter perturbations (Boer et al., 2012). Moreover, although the order in which the parameters where estimated was based on P4 – and therefore indirectly on CL – sensitivities, the predicted peak CL volume was not well adapted to the data points (Figure 6.8 and

6.9). Parameter identification was thus not very successful in predicting the variation in peak CL volume and P4 concentrations, although such variation clearly existed in the raw data. This lack of variation in the model predictions is surprising, because many of the parameters with high sensitivity and low subcondition are involved in CL function and the timing of luteolysis. It could be due to a suboptimal (combination of) parameters that were fitted, indicating that it is important to find the appropriate combinations of parameters to be estimated simultaneously. It was also remarkable that manually changing parameter 49 (proportionality factor of follicular function in Inh increase) did not have a large effect on the simulated curves, although the sensitivity analysis showed that P4 was highly sensitive to this parameter. The first three parameters with the highest sensitivity and the lowest subcondition were involved in luteolytic signaling (Table 6.5). Considering the time point of sensitivity analysis this is not surprising, but it indicates the importance of luteolytic signaling for the regulation of the cycle as a whole.

6.4.3 parameter settings that affect follicular wave pattern

The number of follicular waves was in many cases successfully predicted, but the prediction could be further improved. If the initial parameter setting would have been different from that obtained in Boer et al. (2011b) in that it would still feature a three-wave cycle but with a slower follicle growth rate, it would have been easier (and more biologically plausible) to move to a 2-wave cycle without severely reducing the luteal phase length, as was the case in Boer et al. (2011a). The mean estimated value for par 33 (threshold of P4 to stimulate decrease of follicular function) was twice as high as the initial value (Table 6.2). This suggests that the parameterization of follicular development in the model could be improved, and the current study was a first step. Parameter identification was not successful for 4-wave cycles, possibly due to the way the follicle data is processed in the model. However, a previous study with the model (Boer et al., 2012) showed that the model can simulate cycles with four or more follicular waves. Due to the way follicular function is modeled, simulations with the initial model parameterization clearly distinguishes the separate follicular waves, while the separate follicles are not distinguished. In empirical data, total follicular size does not show a clear wave pattern (Figure 6.2), although obviously there is growth and regression of dominant follicles. In experimental data, a regressing follicle from a previous wave is still visible as a structure, but not functional anymore in the sense of E2 and inhibin production. One way to improve the prediction of follicular function is by optimizing the handling of follicle data. This could be done from two directions: 1) the way in which the data is processed and 2) how follicular function is modeled.

6.4.4 Outlook

This study showed that the model of the bovine estrous cycle is able to identify parameter values based on empirical data of individual cows, without taking extreme, probably non-physiological values. Because the model is capable of simulating 'real' data, it can be used to identify key regulatory parameters that explain variation in estrous cycle characteristics between individuals, and how (the mechanisms underlying) this variation could affect reproductive performance of dairy cows. Prediction of follicular wave pattern was in many cases successful, but could be further improved by adapting the manner in which follicle data is pre-processed. Further development of the model could consist of including an extra equation (with Hill function) to distinguish functional and structural follicular development. Increased steroid metabolism has been suggested as a potential mechanism responsible for reduced circulating P4 concentrations in lactating dairy cows (Wiltbank et al., 2006). For future work, it would therefore be interesting to fit the model parameter of 'P4 clearance rate' and 'proportionality factor of CL function in P4 increase'. Similarly, the proportionality factor and the clearance rate for E2 could be fitted to investigate whether increased steroid metabolism or follicular E2 synthesis can explain variation between animals in peripheral blood P4 and E2 levels. Although it was difficult to detect significant differences in parameter values between groups with different estrous cycle characteristics, the results of this study indicate that specific combinations of parameters can induce a shift in qualitative behavior of the model. This study is therefore a first step towards specific parameter configurations for estrous cycles that vary in characteristics like number of follicular waves or P4 concentrations. Certain combinations of estimated parameter values induce a clear qualitative change in model behavior (e.g., a different number of follicular waves or a change in peak hormone concentrations). This suggests that external or genetic influences on estrous cycle characteristics take place via the mechanisms regulated by these parameters.

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7

General discussion

7.1 Scope of this thesis

In this thesis, we developed and used a mathematical model of the bovine estrous cycle, aiming to improve insight in the regulation of dairy cow fertility. A conceptual physiological model has been translated into a set of mathematical equations. The model represents a computable set of qualitative relationships described in literature. Simulations performed with the model identified candidate physiological mechanisms involved in regulation of the estrous cycle that might affect fertility. The purpose of developing and using this model is to unravel complex biological interactions involved in the estrous cycle. The model delivers 1) quantification of known physiological processes, 2) model predictions for various physiological conditions, and 3) new hypotheses and predictions about regulation of the bovine estrous cycle.

Successful reproduction requires follicular maturation, E2 production, induction of the LH surge, ovulation, and expression of estrous behavior to be coordinated within a tight time frame. Reproductive success, in the period from resumption of cyclicity till fertilization, is determined by two output factors: 1) available egg and 2) estrous behavior. Ignoring egg quality, oocyte releases translates in E2 and P4 profiles, and these two hormones play a crucial role in expressing estrous behavior. For this reason we chose to focus on endocrine markers of follicle and CL development in constructing a dynamic model of the bovine estrous cycle. We emphasized on regulation of the estrous cycle and causes of atypical cycles, which is meaningful as estrous behavior depends on follicle derived estrogens and CL derived P4. A basic model of the estrous cycle is thus prerequisite for prediction of endocrine events regulating estrous behavior. Since it is the combined functioning of several factors that determine the outcome in terms of endocrine and follicular dynamics, it is difficult to distinguish all these in animal experiments. A mathematical model, however, can be used to evaluate these factors simultaneously, especially since the model will not only show the qualitative behavior of e.g. E2 and P4 (i.e. whether the animal is cyclic or not), but also quantitative information (e.g. peak values of E2 and P4). Thus, the endocrine output as a result from organ and cell functioning could be a proxy for estrous behavior. In this way, the mechanistic mathematical model of the bovine estrous cycle that we developed is not only helpful to get an overview of the interplay of factors involved in the current model, but is a basis to increase our understanding of the regulation of estrous behavior. Another reason to develop such a model is to be able to give more precise predictions of the dynamics of the estrous cycle than can be done based on qualitatively described relationships. Mathematical modeling allows to readily study quantitative system performance of the estrous cycle under

many different physiological settings, e.g. follicle growth rate, CL development, etc. One advantage is that such a model can help to identify critical points in the network of physiological events that have a relatively strong impact on fertility.

In this chapter, the results of the previous chapters are discussed from a model-based and a biology-based perspective, and further model applications are proposed. Building, validation and use of the model generated new insights and hypotheses about biological mechanisms that can play a role in declined fertility of dairy cows. It is discussed how a mechanistic mathematical model, next to animal experiments and statistical models, can be helpful to increase our understanding of the complex biological system of the bovine estrous cycle.

7.2 From a model-based perspective

7.2.1 Model extension and reduction: modifications for different purposes

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 components over time. Sets of connected differential equations, allowing simulation of quantitative profiles of the involved components, have been used to model for instance dairy cow metabolism (Baldwin, 1987; Martin and Sauvant, 2007), the human menstrual cycle (Reinecke and Deuflhard, 2007; Harris, 2001) and aspects of bovine reproduction (for example the effect of FSH on follicle growth, Soboleva et al. (2000)). In particular, the model of the human menstrual cycle (Reinecke and Deuflhard, 2007) showed us how to apply a deterministic modeling approach to develop a model of the bovine estrous cycle, using (delay) differential equations with Hill functions to model non-linear stimulating and inhibiting effects. It is important to realize that a model like our model of the bovine estrous cycle is never finished and should be considered as 'under construction', requiring to be updated and extended to improve the quality of its predictions. Models need to be updated when new data, concepts or insights become available, because continued model development improves the understanding of the bovine estrous cycle. One example of this continued model development is the modeling of luteolysis, which developed in the different chapters, back and forth with extra components. Another example is that initially (Chapter 3) time delays were used to obtain a good approximation to the empirical data, but for subsequent studies these time delays were replaced by deterministic descriptions of the underlying mechanisms. A time delay is a black box for a descriptive model, but time delays are very useful to represent certain biological events in a less complicated way. They are used as a simplification of the biological processes or when exact mechanisms are unclear. For this reason the

model that was first published (Chapter 3) contained two long time delays for the effect of P4 on PGF2α. These delays suffice to describe luteolysis as they determine the right time point of this process. However, in subsequent studies (Chapters 4 and 5) it was tried to simulate different physiological settings. For that purpose these time delays, which can be seen as a black box, were too far away from the biological mechanisms to induce relevant physiological changes (described in more detail in 7.4). In the following paragraphs, the process of model development is illustrated with three briefly presented examples of modifications made in the model: simulation of synchronization protocols, modeling of separate follicles, and model reduction.

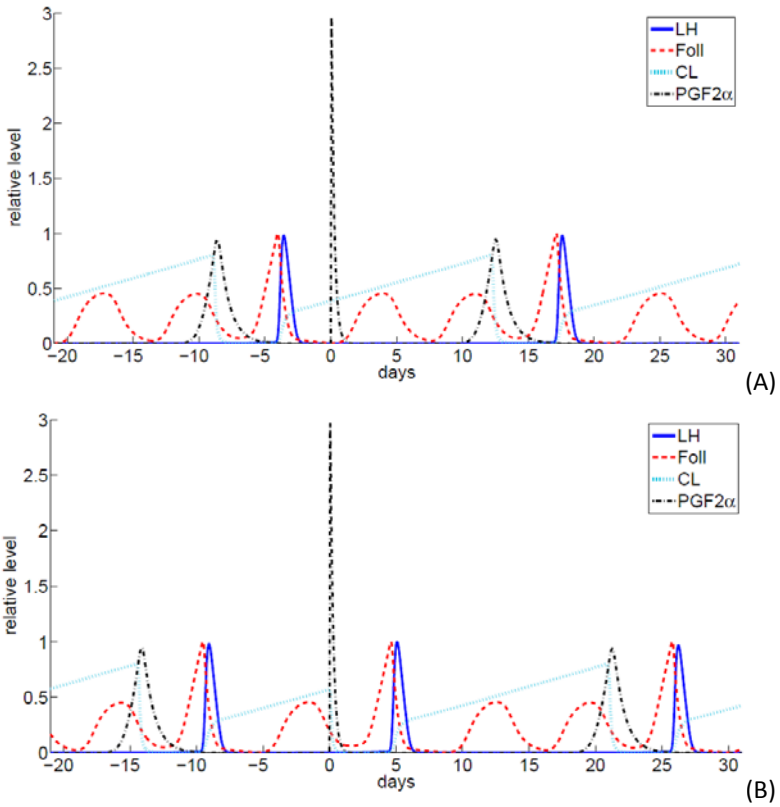

Figure 7.1 *Simulation results for the follicles, CL, and LH for administration of PGF2α on different days in the cycle. Day 0 denotes the day of PGF2α administration. (A) PGF2α administration in the early luteal phase does not lead to luteolysis because the CL is not responsive to PGF2α. (B) PGF2α administration after the first follicular wave leads to luteolysis and to an LH peak within five days after administration (Stötzel et al., 2011).*

7.2.1.1 Simulation of synchronization protocols. Model validation with experimental data is often difficult, due to limitations of the dataset (described in more detail in 7.2.2). Therefore, the model of the bovine estrous cycle was also validated by checking the correctness of model predictions for a specific scenario of which the biological answer is known, i.e. estrous cycle synchronization by PGF2α administration. PGF2α and its analogues are administered to cows mainly using their luteolytic action in estrus synchronization protocols, facilitating timing of artificial insemination. It is known that the sudden rise of PGF2α at certain stages of the estrous cycle results in a fast regression of the responsive CL and hence in decreasing plasma P4 levels. Due to the long time delays in the model described in Chapter 3, the modeling of luteal phase length caused some instability in the model predictions. In Chapter 4, a more detailed modeling of luteolysis (replacing the large delays) was introduced. Further improvement of the model involved the introduction of other new components, like OTR and IOF (Stötzel et al., 2012). The model with the modifications described by Stötzel et al. (2012) was validated by showing that the simulations agree with observations from estrous cycle synchronization studies and with experimental data of P4 after a single dose administration of synthetic PGF2α. PGF2α administration was conducted at various days of the estrous cycle. Simulation results were in good agreement with available experimental data and biological knowledge. PGF2α administration in the early luteal phase does not lead to luteolysis because the CL is not responsive to PGF2α. PGF2α administration after the first follicular wave leads to luteolysis and to an LH peak within five days after administration (Figure 7.1, see also Stötzel et al., 2012 for more examples). The agreement is remarkably well, especially when one considers that the equations and parameter values of the model were derived primarily from qualitative data. This also shows that absolute levels are less relevant for model performance. The model must be quantitative to show underlying interactions and dynamics. The relationships between model components are important, while the absolute values have less importance.

7.2.1.2 Multiple follicles – including stochastic elements. The model as described in Chapter 3 is entirely deterministic and thus involves no probabilistic processes. Stochastic elements can be included in such a deterministic model when biological processes cannot simply be modeled by use of ordinary differential equations. For example, in the model of the human menstrual cycle (Reinecke and Deuflhard, 2007) a stochastic approach was chosen to model pulsatile release of GnRH. In a study by Bondouy (2011) parts of a model for follicle development in sheep and cows (Smith and Soboleva, 2005; Soboleva et al., 2000) were used to add stochastic

fluctuations to the model of the bovine estrous cycle. A normal bovine estrous cycle consists of 2 or 3 waves in which a cohort of follicles start to grow. In the model described in Chapters 3-6, 'follicular function' stands for the total amount of hormone production of the follicles present at any moment. This is a valuable representation of follicular waves, but does not provide information about growth and development of single follicles. Instead of modeling the growth of follicles by using one differential equation, in the multiple follicle model one equation is used for each follicle. The number of follicles developing per wave is determined by a fixed parameter value and stochastic initial values determine which follicle becomes dominant. This gives the possibility to model the influence of the dominant follicle on the other follicles. Also the emergence of a new follicular wave was initialized in a stochastic way. With different numbers of follicles this model with stochastic elements simulates cycles of approximately 21 days with three waves of follicular development (Figure 7.2). Including these stochastic elements is a first approach in modeling each follicle separately. Modeling follicles separately is a better representation of biological data, and could identify candidate physiological mechanisms that explain follicle dominance. Furthermore, it allows to model follicular E2 and Inh production in more detail, which could be helpful to investigate for instance E2 requirements to induce the GnRH/LH surge and estrous behavior.

7.2.1.3 Model reduction. Reduction of the number of equations and parameters of the model and still generating the output required for its functional behavior is a greater challenge than improving the model by including more details. It is always the objective of modelers to predict with the lowest number of parameters, although obviously one must always check whether the mechanisms in the reduced model are still biologically plausible. The question is which model components are essential for the predictive power of the model. For our model of the bovine estrous cycle, the complexity reduction method described by Apri et al. (2012) was used to simplify the model. This method uses the sensitivity of the parameters as guideline to find out which parameters could be removed. It investigates whether model components (for which a differential equation is derived) could be omitted or lumped (i.e. two components are replaced by one) while the model outcomes still match the output data (within some given range). By removing a component, also its reactions disappear, which reduces the number of parameters. With the method of Apri et al. (2012) the model could be reduced from 15 to 10 equations (Figure 7.3) and from 60 to 45 parameters. One simplification was to omit OTR and OT from the model. Another simplification was to lump the effects of the

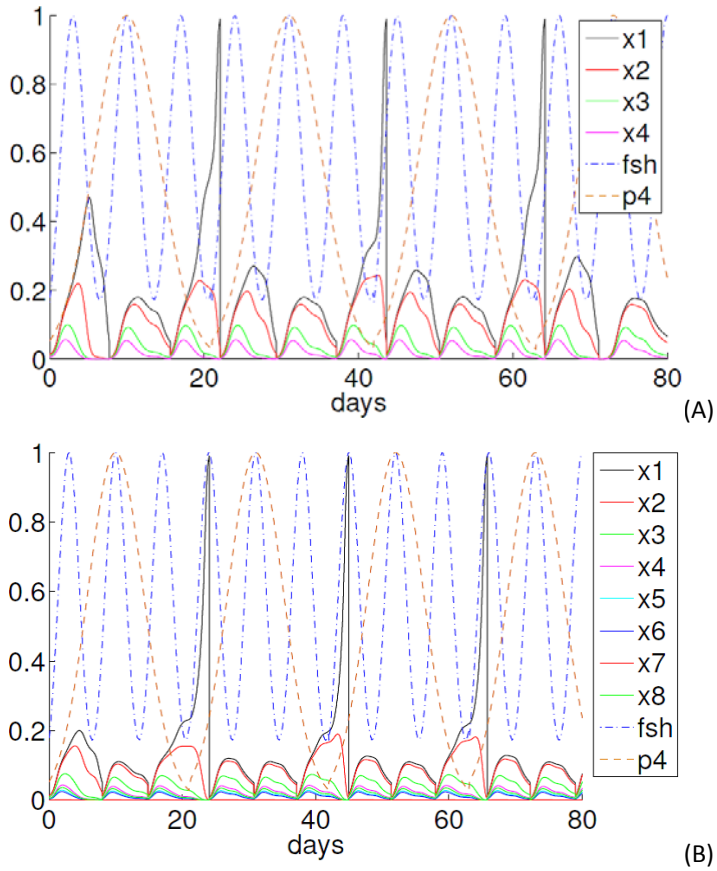

Figure 7.2 *Simulation results of the model with the modifications described by Bondouy (2011) using stochastic processes for follicular wave emergence and development of single follicles. (A) Simulations for four follicles (x1-x4) and (B) Simulation for eight follicles (x1-x8), (Bondouy, 2011).*

hypothalamus and the pituitary together and consider the synthesis and release of FSH respectively LH in a single equation, which was also done in the model of the human menstrual cycle described by Margolskee and Selgrade (2011). Both models (the one with 15 and the one with 10 equations) produce very similar output curves with their initial parameterization. A risk of model and parameter reduction is that some effects or mechanisms, which in the extended model could be studied, can no longer be addressed in the reduced model. Although the reduced model still generates the output that is prescribed to a normal cycle (for the dynamics we are interested in at that moment), it is yet not known how the reduced model will behave when different physiological settings are simulated. It could well be that details are lost that affect its predictive power. For instance, the options to study

many variables of OT and OTR effects are blocked, because in this reduced model OTR and OT are omitted. Therefore, when using the reduced model one should keep the omitted factors in mind in case one needs to study certain specific effects.

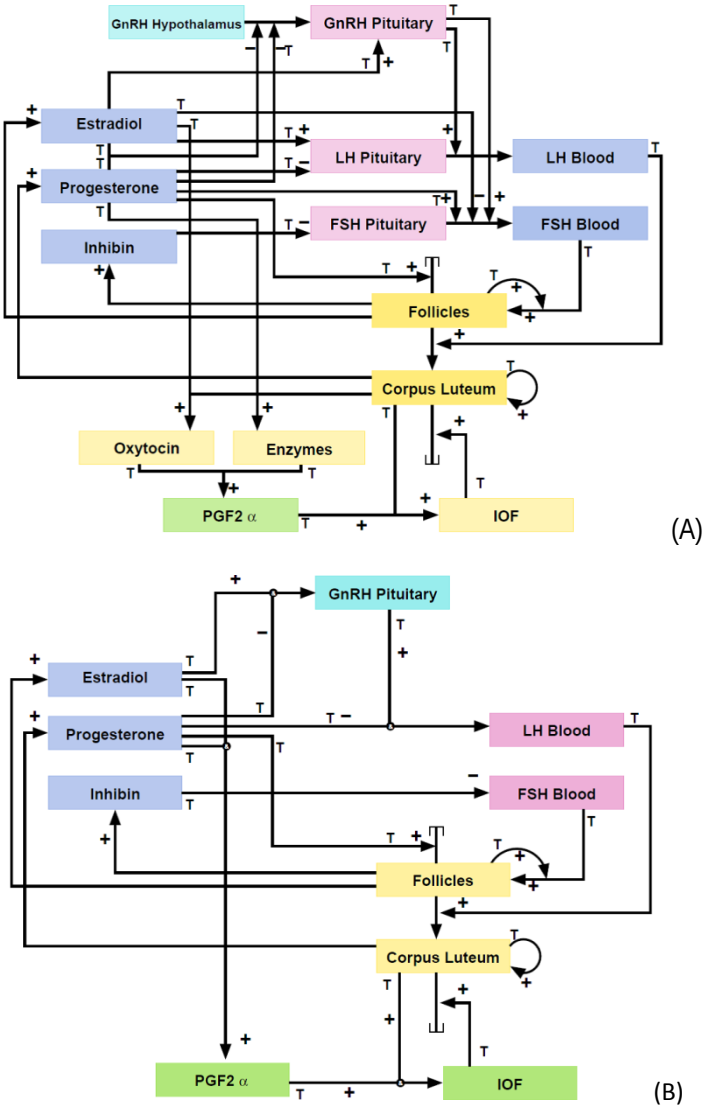

Figure 7.3 *Comparison of the full and the reduced model. (A) Flow chart of the full model with 15 equations and 60 parameters. (B) Flow chart of the reduced model with 10 equations and 40 parameters. Boxes represent the components for which a differential equation was derived. At the arrows (indicating specific parameterizations of a given equation), stimulating and inhibiting effects are denoted by + and -, respectively.*

These three examples (synchronization protocols, multiple follicles, and model reduction) are not just modeling exercises, but also a way to identify and explore the key components of the system. Possible sources of model errors are the assumptions and simplifications, but the model itself is just a way to formalize existing assumptions in a quantitatively verifiable manner. Every model mechanism is 'lumped' biological knowledge, so nothing new is 'invented' by the modelers. However (as will be addressed in 7.3), based on the model predictions new hypotheses and ideas about regulation of the estrous cycle (see e.g. Chapter 4) and the cause of atypical cycles (see e.g. Chapter 5) are formulated. The benefit of the model is that it shows how different known mechanisms work together to produce phenotypic traits. Based on only qualitative assumptions about separate model components, one can only poorly predict the functioning of the system. The advantage of developing and using this model of the bovine estrous cycle is to control and quantify qualitative assumptions. Thus, new findings on system performance are generated from a set of known relationships. However, these intuitive qualitative assumptions are only successfully predicted in a mathematical sense when the model is properly parameterized. If the model parameterization is of poor quality the simulation results will show behavior that does not make sense, although the model in itself may not be wrong.

7.2.2 Validation – building confidence in the model

Chapters 4-6 can be seen as steps in model verification (does the model behave as intended) and validation (does the model behavior agree with the behavior of the real biological system) (Sørensen, 1990). A mathematical model is considered 'good' if it is able to describe and predict the dynamic behavior for which it has been developed, but correct predictions are only possible when the model is properly parameterized. Validation with experimental data is important to develop the descriptive model (Chapter 3), towards a predictive tool that is numerically confident, can be used to predict various abnormalities in estrous cycles, and can predict estrus characteristics for individual cows based on some measured parameters. There are various approaches of model validation. The most important criteria 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. In this paragraph, four steps are discussed that are considered to be important for the model building and validation of the model of the bovine estrous cycle described in this thesis: parameter identification, sensitivity analysis, stability, and model predictions for different scenarios.

Identification of involved parameter values is the main difficulty in solving the system of differential equations. This is particular difficult because most parameter values in the model are neither measurable or perhaps measurable but not yet available in the literature. 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. Another difficulty in parameter identifiability is that different parameter values may be equally consistent with the data. Under these circumstances there is no unique solution and estimating all parameter values (i.e. calibration) simultaneously is impossible. Therefore, we used a model decomposition approach to obtain a first estimate of the parameter values. 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 (Chapter 3). A subsequent aim was to find parameterizations of the model that give a good fit of the output data with measurement data of individual cows, as described in Chapter 6. Data collected for other reasons than validation of the model will typically not have all the variables needed. Therefore, model validation with experimental data published in literature is often difficult. Measurements often do not meet the requirements for individual parameterizations, because for example the observed time scales are too small or too coarse, or too few substances are measured. Nevertheless, testing the goodness of fit of simulated output with observed data is an important part of the process of assigning parameter values to complex mathematical models (Chapter 6).

Sensitivity analysis provides insights about which model parameters are the key factors that affect simulation outputs and about model robustness with respect to changes in parameter values. A higher sensitivity means that a change in the value of the parameter has a stronger 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 parameter estimation, but also in model validation, since it quantifies the relative importance of parameters. Thereby it identifies if the model depends unexpectedly strong on presumably biologically less relevant parameters. In Chapter 3, a sensitivity analysis for the complete set of model parameters has been performed with techniques described in Deuflhard (2004). 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. The parameters with higher sensitivity are more likely to provide useful biological insights than those with lower sensitivity, although it is important to realize that sensitivity of parameters is not only influenced by the biological system but also by the structure of the model itself.

Model validation also deals with stability. Stability relates to how changes in the model input affect model output. In a stable model, small perturbations should not disturb the qualitative behavior of the system (unless it is known that this is the biological reality). Some parameterizations of our model produce a stable limit cycle (periodic behavior, see for example Figure 4.3), while others generate consecutive estrous cycles that are not entirely identical (quasi-periodic behavior, see for example Figure 3.5b). The variations between simulated cycles are therefore 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 that in real cows, 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 is also true in our model. Thus, the level of instability that may be found, depending on the parameterization, would be a biologically relevant aspect of our model. A certain level of stability of the model is, however, an essential requirement to handle variation between individuals.

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 (e.g. the follicular wave patterns described in Chapter 4), or by mimicking for instance external hormone administration (e.g. the synchronization protocols described by Stötzel et al. (2012)). Such model simulations of different physiological settings help to understand the overall structure and dynamic behavior of the model and thereby enhances the confidence of the user. 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. 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.

7.3 From a biology-based perspective

7.3.1 From model to experiment, and back

7.3.1.1 The modeling cycle. Theoretically, there are many possible parameter configurations that obtain a biologically realistic output for a model like the one described in this thesis. In such complex models, initially, the reliability of the model predictions is more important than the exact parameter values, since the true (empirical) parameter values are difficult to determine. During the process of model development an iterative process between experimental measurements and parameter identification, also known as the modeling cycle (Potter and Tobin, 2007), is required to determine all control parameters. However, large-scale dynamic mathematical models often suffer from missing data for parameter identification (as discussed in 7.2.2 and in Chapter 6). Using our model we identified processes that play an important role in the regulation of the bovine estrous cycle according to our simulation results. These processes could be investigated in animal experiments. The question is which system components should be measured in which time interval to increase the number of identifiable parameters. Possibilities for identification are closely related to parameter sensitivity (e.g. the value of a parameter with low sensitivity could be difficult to identify because many different values will give the same model output). In cyclic systems the sensitivity has to be considered throughout a whole period (i.e. at all time points of a cycle). In particular, the influence of parameters on the cycle length must be considered. Therefore, data averaging over measurements from individuals with different cycle lengths needs to be reconsidered. Understanding variation in simulation results due to changes in specific parameters may help to construct biological interpretations (discussed in 7.3.2) from model behavior, which indicate directions for future biological experiments. The simulation results described in Chapters 4 and 5 show some likely candidates involved in the regulation of follicle wave patterns and P4 profiles that could be tested in future experiments. Further refinement of model parameterization based on critical experimentation is required. These experiments require daily measurements of follicle and CL dynamics and blood sampling for successive cycles.

7.3.1.2 An illustration of the modeling cycle: improved understanding of luteolysis

The completeness of description demanded by computational models highlights gaps in knowledge and points of uncertainty that have received little study, like the mechanisms involved in PGF2α induced luteolysis. The modeling of luteolysis is a nice illustration of the modeling cycle (Figure 7.4). In Chapters 3 as well as 4, 5, and 6 luteolysis appears to play a crucial role in the regulation of the cycle. The time point of luteolysis affects the length of the estrous cycle directly and therefore

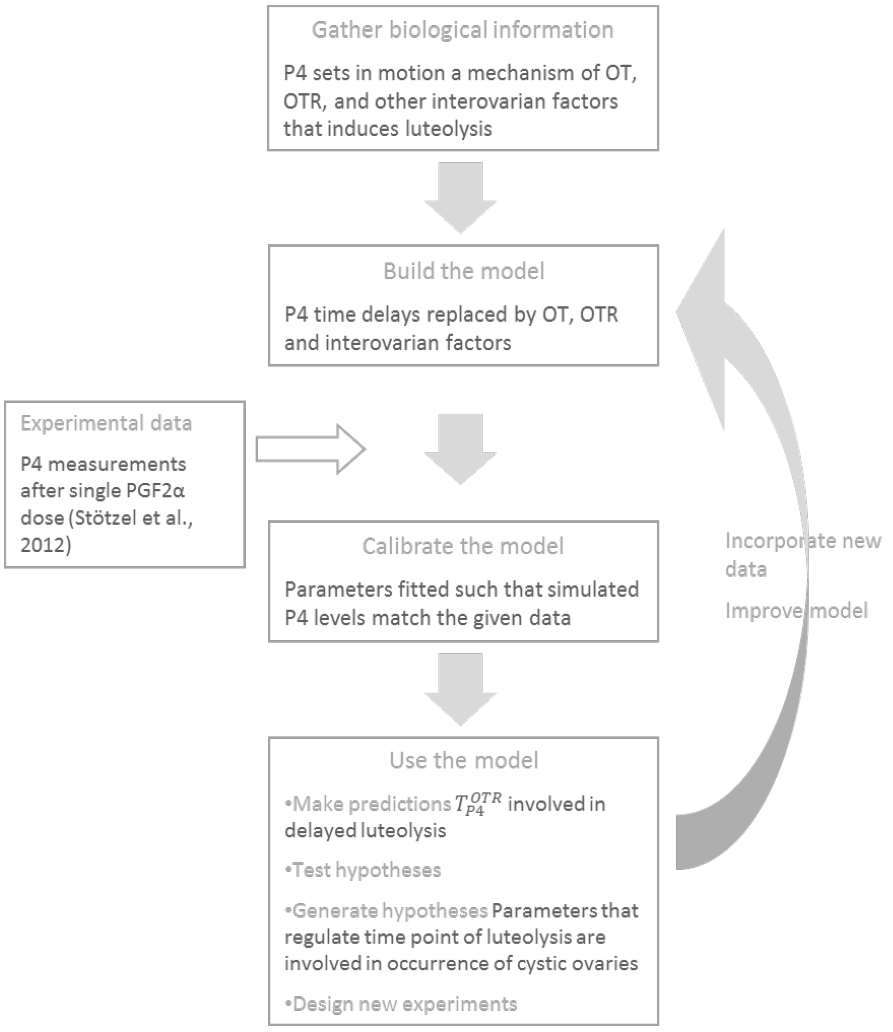

Figure 7.4 *Modeling cycle (adapted from Potter and Tobin, 2007) with modeling of luteolysis as an example.* T_{P4}^{OTR}*: threshold of P4 to stimulate OTR increase.*

influences all other curves as well. In the model described in Chapter 3 PGF2α increases a specific number of days after P4 levels reach a threshold. Similarly, PGF2α decreases another (larger) number of days after P4 levels reach a threshold. The right timing of growth and decline of PGF2α to induce luteolysis was thus modeled using time delays, representing that continued presence of P4 above an effective level sets in motion a series of events that eventually lead to luteolysis. These two time delays of P4 are very sensitive model parameters. Using time

delays in modeling is questionable from a biological point view. Therefore, in Chapter 4 the 'black box' of the intermediate events that regulate PGF2α is modeled in a bit more detail by replacing the time delays by a mechanism in which the ability to synthesize PGF2α develops over time under influence of P4. P4 stimulates the synthesis of receptors (e.g. OTR) and enzymes required for the production and release of PGF2α. In the model described in Chapter 4 OTR represents the overall mechanism in the endometrium involved in the production of PGF2α. PGF2α levels thus increase because P4 stimulates the production of OTR required for PGF2α production. A decrease of the threshold of OTR above which the stimulating effect on PGF2α is increased appears to induce a change from a 3-wave to a 2-wave cycle, and this parameter involved in luteolysis is thus important for the regulation of the follicular wave pattern. In Chapter 5 the modeling of luteolysis was further specified by introducing (luteal) OT next to OTR as stimulator of PGF2α production. The effect of PGF2α on the CL is mediated by several local factors, such as endothelin-1-system, cytokines, and nitric oxide, included in the model as interovarian factors (IOF) (Skarzynski et al., 2008). This 'IOF' is included to prevent that PGF2α would have a too early effect, because it is known that the CL is not responsive to PGF2α in the early luteal phase. This model modification improved the simulation results for estrous cycle synchronization with PGF2α (7.2.1.1). The simulation results described in Chapter 5 suggest that in the real cow the threshold of P4 to stimulate OTR increase (among other parameters) strongly determines the occurrence of P4 patterns associated with delayed luteolysis in the model. In Chapter 6, parameters of the model described in Chapter 5 were fitted to experimental data. Here, P4 appears to be very sensitive to changes in the threshold of OT to stimulate PGF2α increase, and large variation between cows was found for the estimated value of this parameter. Also given this large variation between cows, it is hypothesized that *in vivo* measurements of P4 levels at which OTR and other luteolytic factors start to increase could indicate which cows are prone to cyst development.

Thus, in Chapter 4 as well as 5 and 6 a threshold value involved in the regulation of luteolysis was indicated as important for the dynamics in the estrous cycle. The reason that in the three different chapter three different thresholds are identified as being important is the difference in modeling of luteolysis. However, the results in all three Chapters suggest that the P4 induced switch in OT and OTR production, preceding the actual PGF2α release, exert a high level of control on the time point of luteolysis for the cow. The reason why the mechanism of luteolysis was initially modeled as a 'black box' is that luteolysis is preceded by a complex system of interactions and feedback mechanisms that is known to result ultimately

in CL regression, but that is not understood in full detail. Recent publications (e.g. Ginther et al., 2011) show that current research is focused on the small time frame around the start of luteolysis, without investigating the events in the days preceding luteolysis. What is clear is that sequential PGF2α pulses (via a local utero-ovarian pathways) are necessary to induce natural luteolysis in cows and that a single injection of a larger dose of PGF2α (as applied in estrous cycle synchronization protocols) can also induce luteolysis (Ginther et al., 2009). However, how the exact time point of spontaneous luteolysis is regulated in untreated cycling cows is not fully elucidated. A reduced prominence of PGFM pulses (the main metabolite of PGF2α) during luteolysis delayed completion of luteolysis (Pugliesi et al., 2011). Model simulations that mimic this delayed completion of luteolysis could be used to optimize the modeling and parameterization of luteolysis. Model simulation results were thus helpful to identify gaps in knowledge, and thereby help to optimize experimental design, e.g. to investigate the dynamics of OT and OTR that precede luteolysis, and the relation of OT and OTR with P4 levels.

7.3.2 Current ideas about subfertility in dairy cows

7.3.2.1 Cystic ovaries. Cystic ovaries are an important cause of reduced reproductive performance, as ovarian cysts clearly interfere with normal ovarian cyclicity and are frequently diagnosed in dairy cattle (Chapter 5). The collective term 'cystic ovaries' represents a not strictly defined variety of situations with follicles that differ in production of E2 and where luteolysis can take place or not (Vanholder, 2005). It is challenging to simulate and predict such a messy system with the model in a biologically plausible way. Nevertheless, the results described in Chapter 5 shows transitions from one stable (normal) cycle to another in which irregular cycle patterns occurred. The variety of histological and endocrine conditions during cyst development and maintenance explains why the exact etiology of cystic ovary syndrome has not been clearly established. Currently, it is generally accepted that cystic ovaries result from imbalance of the hypothalamic-pituitary-gonadal axis. Since no clear differences have been found in systemic levels of reproductive hormones (Vanholder et al., 2005; Probo et al., 2011), cyst development and maintenance is more likely the result of local effects than of systemic influences of reproductive hormones. Yet, systemic IGF-1 levels have been associated with the development (Vanholder et al., 2005) and the maintenance (Probo et al., 2011) of cystic ovaries in cattle, although the exact mechanisms by which IGF-1 is involved in cyst formation are not elucidated. Likely, the systemic changes in IGF-1 are a cause or a representation of local ovarian effects.

Since model parameters could be a good representation of local effects of certain substances, model simulations are very useful to generate hypotheses concerning these effects. For example, the parameter that represents the maximum CL growth stimulated by itself will likely involve local effects. Delayed ovulation, which indicates cystic follicle formation, occurred in the model in absence of an LH surge (Chapter 5). In the simulation results described in Chapter 5 delayed ovulation (as defined based on P4 levels) was associated with low E2 (as well as low P4) levels, which according to Roth et al. (2012) would indicate a persistent follicle. Dysfunction of the positive feedback of E2 on LH could precede ovulation failure and formation of a follicular cyst in the early postpartum period (Roth et al., 2012). Simulating this mechanism of dysfunction of the positive feedback of E2 on LH by manually changing certain model parameter values can therefore be used to define hypotheses about the underlying mechanisms of follicular cyst development and help to design experiments to test these hypotheses *in vivo.* A number of model parameters (Table 7.1) could, based on biological knowledge, be involved in this feedback mechanism of E2 since these parameters are all involved in the effect of E2 on LH release. The multi-parameter perturbations presented in Chapter 5 shows how large perturbations can be before the boundary of the so-called robustness region is reached. According to Figure 5.6, perturbation direction 4 has a relatively short distance to the boundary of the robustness region. In this perturbation direction, the parameter 'Threshold of E2 to suppress GnRH release' (involved in E2 inhibited GnRH release during the luteal phase) is most perturbed, suggesting that this parameter determines the occurrence of P4 patterns that are associated with delayed ovulation and delayed luteolysis in the model. Comparison of hypothalamic E2 and P4 receptor expression between healthy and cystic cows could indicate if this process indeed plays a role in cyst development.

Table 7.1 *Model parameters involved in the effect of E2 on LH release, which possibly play a role in cyst development.*

Model parameters involved in effect of E2 on LH release
Threshold of E2 to increase pituitary sensitivity for GnRH
Maximum scaling of pituitary sensitivity for GnRH
Threshold of E2 to suppress GnRH release
Threshold of GnRH to stimulate LH release
Maximum part of LH release rate that is stimulated by GnRH
Threshold of E2 to stimulate LH synthesis
Maximum part of LH synthesis that is stimulated by E2

Effects of altered gene expression could be represented by altered model parameterization (Table 7.2). A comparison of gene expression in ovarian granulosa cells between normal dominant and cystic follicles indicated a number of genes that may be related to the persistence of follicular cysts (Grado et al., 2011). For example, *ANG* (angiogenin) mRNA was found to be up-regulated in cystic estrogenic follicles compared to non-cystic estrogenic follicles. It was therefore suggested that an increased vascularity may be part of a mechanism by which cystic follicles are able to prolong their lifespan. Also *PTGER4* (prostaglandin E_2 receptor 4) was up-regulated in cystic compared to normal follicles. Since PGE_2 plays an important role in the regulation of ovulation, luteinization, and luteolysis, this could indicate that a disruption in these mechanisms plays a role in cyst formation. A transcript similar to *IHH* (Indian hedgehog protein precursor) was down-regulated in cystic estrogenic follicles compared to non-cystic follicles. Studies in mice, where *IHH* expression in granulosa cells decreased after the LH surge, suggest a role for *IHH* in the differentiation of granulosa cells towards P4 producing cells after ovulation (see Grado et al. (2011) for references). The exact role of *IHH* in cyst formation in cows is not elucidated, but it could play a role in cyst formation because it is involved in signaling in advanced stages of follicle development. A transcript similar to *SFRP4* (secreted frizzled-related protein 4 precursor) was down-regulated in cystic compared to non-cystic follicles, and it was suggested that *SFRP4* plays a role as modulator of WNT signaling in granulosa cells of periovulatory follicles. *SFRP4* could therefore be involved in differentiation of granulosa cells towards P4 producing cells (Grado et al., 2011). The genes mentioned above are involved in local effects on CL development and maintenance, and could therefore be captured in e.g. the parameter for 'maximum increase of CL stimulated by CL itself' or in other model mechanisms. Their effects could be included in the model (first by means of different parameterization) to study their role in cyst development. Down- and up-regulation of specific genes could then be represented by a lower or higher parameter value respectively for the process they affect. Altered model parameterization aiming to mimic effects of gene expression could generate hypotheses that could be tested in a further advanced stage of the model where expression of specific genes is incorporated as additional model components. Genes could be linked to processes that were indicated to affect the occurrence of cystic ovaries by the simulation results of the model. Further development of the model could include specific parameters that represent the level of expression of these genes.

Table 7.2 *Genes differentially expressed in cystic follicles (Grado et al., 2011) and their possible effect on the occurrence of delayed ovulation and/or delayed luteolysis in the model by means of a change in specific model parameters.*

Differentially expressed gene in cystic follicles (Grado et al., 2011)	Represented by model parameters	Possible effect
ANG (angiogenin) up-regulated	Parameters related to follicular growth	Follicles prolong their lifespan by increased vascularity
PTGER4 (prostaglandin E_2 receptor 4) up-regulated	Parameters that regulate follicular response to signals that induce regression	Disrupted ovulation due to changed PGE_2 receptor expression
IHH (Indian hedgehog protein precursor) down-regulated	Parameters that induce the start of CL development	Hampered differentiation of granulosa cells to P4 producing cells
SFRP4 (secreted frizzled-related protein 4 precursor) down-regulated	Parameters that induce the start of CL development	Hampered differentiation of granulosa cells to P4 producing cells

7.3.2.2 Other effects on fertility. Subfertility in modern dairy cows is a multifactorial problem. Interactions between factors related to for example negative energy balance, stress, and poor expression of estrous behavior reduce reproductive performance. Likely, the demands of high milk production have a detrimental effect on physiological factors involved in fertility (Walsh et al., 2011; LeBlanc, 2010; Evans and Walsh, 2012). Stressors such as lameness and heat stress affect circulating hormone levels and reduce the intensity of estrous behavior (see Chapter 2, and more recently reviewed in Walsh 2011) and lead to subfertility. In this respect, the mathematical model of the bovine estrous cycle is very useful because it can simultaneously predict the effects of various factors. Furthermore, the model was used to simulate differences between cows. Cows differ in e.g. the amount of E2 that is required to induce estrus. A positive correlation between E2 concentration and duration of estrus in Holstein cows that were treated with different doses of E2 was found by Reames et al. (2011), who hypothesized that there is a threshold of E2 to induce estrous behavior and that additional E2 increases duration and intensity. Peak E2 concentrations in modern high milk producing dairy cows are lower than levels observed during the 1970s, and concurrently the duration of estrus is considerably shorter (see Reames et al. (2011) for references). The high incidence of ovulation without expression of estrous behavior in modern dairy cows may be explained by the ability of low doses of E2 to induce an LH surge but not estrous behavior (Reames et al., 2011). It was indeed predicted by the model that ovulation (represented by a sudden decrease in follicular function) takes place after LH surges with only low amplitude, since these

surges were also followed by the start of a new cycle. If indeed a higher metabolic rate reduces circulating levels of E2 (as hypothesized by Wiltbank et al., 2006), this could have a detrimental effect on fertility due to a disconnection between estrous behavior and the LH surge. Both in ewes and cows (see Reames et al. (2011) for references) it has been observed that low doses of E2 delay the onset of estrous behavior but not the LH surge, which could result in inseminations at a later time point than the optimum. An LH surge sometimes occurred in cows that were not detected in estrus, while estrus was never observed in cows that did not have an LH surge (Reames et al., 2011). This suggest that the hypothalamus, one of the brain areas that regulate behavior, is more sensitive to E2 regarding regulation of the LH surge than regarding regulation of estrous behavior. The difference in hypothalamic response to E2 in regulating the expression of estrous behavior and the secretion of LH is easy to account for in the model parameterization, which makes the model a helpful tool to explore this, once behavior is included in the model as such. Model simulations could be used to identify biological mechanisms that are possibly responsible for this variation between individual cows.

Short exposure of the ovulatory follicle to P4 during its growing phase (as occurs in 3-wave cycles) was in beef cows not associated with higher pregnancy rate than a longer P4 exposure (as occurs in 2-wave cycles) (Dias et al., 2012). However, differences in diameter of the ovulatory follicle were reported between short and long P4 exposure groups and between cows and heifers, and it could be investigated with the model which parameters possibly cause these differences. Model simulations (Chapter 4) already showed that a change in the parameters that affect the number of follicular waves per cycle also induced a change in follicular size. Low numbers of follicles larger than 3 mm in diameter were associated with poorer reproductive performance in dairy cows, represented by for example longer calving to conception interval and lower pregnancy rate at first service (Mossa et al., 2012). Simulations with the multiple follicle model (7.2.2) could generate hypotheses about the underlying differences that cause this effect of number of follicles on reproductive performance. The association between low number of antral follicles, higher FSH secretion and lower P4 production was suggested to result in increased rates of embryo mortality (Mossa et al., 2012). Although fertilization and embryonic growth are not captured by the model, using the model to perform simulations under different physiological settings can provide relevant information on potential fertility of a cow by evaluating the predicted values for FSH and P4.

7.4 Outlook – recommendations for future research

The model developed in this thesis focused on interactions in the hypothalamic-pituitary-ovarian axis controlling the dynamics of the bovine estrous cycle. By constructing, testing, and using this model, our understanding of critical physiological mechanisms underlying the bovine estrous cycle has been increased (e.g. luteolytic signals, as described in 7.3). Development of the model not only identified gaps in knowledge and new hypotheses, but also gave inspiration to study other mechanisms and parameters that were revealed during model simulations.

Although the model described in this thesis performs adequately for the regulatory mechanisms of the hypothalamic-pituitary-ovarian axis, further developments and model extensions will be helpful to investigate other aspects of the estrous cycle. Simulations with various parameter configurations already show when a cycle is disturbed and what the physiological reason for this irregularity could be, although the causal factor of that irregularity, e.g. negative energy balance, is yet not included in the model as such. The ultimate purpose is to develop a model that can be used to predict reproductive performance across a wide range of genotypes and management environments. The current model at cow level could be integrated at herd level and even at livestock system level, but also the genetic level could be modeled in more detail in order to understand links between gene expression and physiology. Many further simulations and applications with the model described in this thesis are possible, but two possible model extensions deserve special attention: energy metabolism and estrous behavior.

7.4.1 Energy metabolism

Reproduction is affected by nutritional state. High milk production affects energy metabolism, which can disturb endocrine signaling (Chapter 2). It is therefore interesting to merge the estrous cycle model with existing metabolic models, as a first step towards the integration of reproductive processes and nutrient fluxes. Examples of existing metabolic models are the 'Molly' model (Baldwin et al., 1987) that describes the supply and partition of nutrients in lactating dairy cows, models focusing on rumen metabolism (e.g. Bannink et al., 2011; Dijkstra et al., 1992), and the model of Martin and Sauvant (2007) that integrates the mean kinetics of body weight changes as a result of feed intake and milk production. A better understanding of the interplay between nutrition and fertility can lead to new concepts for breeding, nutrition and management of dairy cows.

Metabolic components are yet not part of the model, but some interesting metabolic effects can be hypothesized already. For example, fatty acid composition of the diet can affect dairy cow reproduction via alterations in follicular and CL function, P4 secretion and estrous behavior. Cows that were fed a diet supplemented with extruded flaxseed had a longer duration of high E2 levels and exhibited longer duration and greater intensity of estrous behavior than control cows (Zachut et al., 2011). Effects of different source of fat supplementation on hepatic and endometrial gene expression were studied by Hutchinson et al. (2012), who reported differential effects of the fat supplements (flaxseed, CLA, fish oil) on P4 levels and CL volume. Flaxseed supplemented cows demonstrated increased peak E2 levels, which potentially can increase the duration and intensity of estrous behavior. Plasma IGF-1 has been shown to be highly correlated with E2 concentrations (for references see Hutchinson et al., 2012). It could be evaluated by which parameter configurations such metabolic effects are most easily simulated by the model, which could indicate a likely biological mechanism.

Instead of just changing a few parameter values to mimic such metabolic effects, one could introduce and extra model equation that represents these effects, which will allow to study the dynamics of this process in more detail. As a start, the current model of the bovine estrous cycle could be extended with known effects of insulin, IGF-1 and glucose. Including extra model components and equations not only allows to predict metabolic effects in more detail than by just changing a few parameter values, but it also can simulate the dynamics of metabolic effects in a biologically more sound way. For example, IGF-1 levels might have an effect only when the cow is in negative energy balance and not during the rest of the lactation. The model simulations could be corrected for this effect when the estrous cycle is a function of energy intake and the lactation curve. New model components are also helpful to deal with time intervals, e.g. because negative energy balance will affect primordial follicles that become an ovulatory follicle much later. Next to effects of IGF-1, insulin, leptin, growth hormone, etc. on normal cyclicity, resumption of cyclicity after parturition is highly depending on energy balance. Effects of energy metabolism should be taken into account when one wants to model not only normal cyclicity but also a broader period of the cows reproduction cycle.

7.4.2 Estrous behavior

Like the estrous cycle in general, expression of estrous behavior can be influenced by many factors, like genetics, milk production and health status (reviewed in Roelofs et al., 2010). The detection of estrus in modern high milk-yield dairy cows is

hampered, because the duration and intensity of estrous behavior in these cows is considerably lower than that in dairy cows of a few decades ago (reviewed by Lopez et al., 2004). Estrus detection is therefore a limiting factor for reproductive performance in modern dairy cows. Hence, it would be interesting to include estrous behavior in our mathematical model of the bovine estrous cycle. A number of factors that are known to be involved in the regulation of estrous behavior (e.g. E2, OTR and OT) are already in the model, and thus provide a starting point by which estrous behavior could be predicted by the model as well. For instance, a correlation of 0.7 was found between maximum E2 concentration prior to estrus and intensity of estrous behavior (Lyimo et al., 2000). Additionally, presence and decline of P4 has an important role in priming of estrous behavior (see Chapter 2 and Woelders et al. (2012) for references). Expression of estrous behavior could thus already be predicted by the model based on E2 and P4 profiles, i.e., when the optimal endocrine profile is specified, the model outcome can be interpreted in terms of predicted low or high expression of estrous behavior.

E2 increase stimulates activation of estrous behavior, but E2 levels explain only a small part of the variation in estrous behavior expression between cows. The model could be extended by including mechanisms that explain how ultimately estrous behavior is elicited at given elevated E2 levels. This could explain variation in estrous behavior expression in response to E2. There are for instance differences in the response of behavioral and secretory centers in the hypothalamus (which is one of the brain areas important for regulation of behavior) to E2. The neurological basis for these differences warrants further investigation (Reames et al., 2011). Estrous behavior occurs around the same time as the preovulatory GnRH/LH surge, making it difficult to distinguish independent neuronal control mechanisms. The ventromedial nucleus (VMN) and arcuate nucleus (ARC) of the hypothalamus contain the major sites of action for E2 to induce both the GnRH surge and estrous behavior, and also contain insulin receptors and play a pivotal role in glucose-sensing. Therefore, it is likely that specific types of neurons located in these hypothalamic areas are altered by insulin administration in the late follicular phase, resulting in disruption of the GnRH surge (Fergani et al., 2012). Also cortisol activity may play a role in this hypothalamic signaling, as insulin-induced hypoglycemia is a potent stressor and has been shown to delay the LH surge in ewes, while the timing of estrous behavior was not affected (Saifullizam et al., 2010). Estrous behavior in ewes was delayed after acute insulin administration, while duration and frequencies of the various behavioral signs of estrus did not differ (Fergani et al., 2012). This suggests that these behavioral signs have a common regulating factor,

presumably E2. However, the fact that they began and ended sequentially suggests that these behaviors also have independent controlling mechanisms.

In a study of Cutullic et al. (2012) cows were assigned to two feeding level groups: high feed, resulting in high milk yield and moderate body condition loss, and low feed, which limited milk yield and triggered a large body condition loss. Thereby, the effects of milk yield and body condition on reproductive performance were less confounded. It was suggested that estrus cyclicity is mainly influenced by body lipid reserves, whereas the expression of estrous behavior is mainly influenced by milk production (Cutullic et al., 2012). Another important factor in the regulation of estrous behavior is P4 priming (reviewed in Roelofs et al., 2010). P4 increases the number of estrogen receptors in the hypothalamus during the luteal phase. Consequently the sensitivity of the hypothalamus to E2 is increased, which can have positive effects on the intensity of estrous behavior. Also prostaglandin regulators appear to play an important role in the expression of estrous behavior (Woelders et al., 2012). This illustrates the importance of prostaglandin signaling in the dynamics of the estrous cycle regarding the synchronization of estrous behavior and ovulation (by determining the time point of luteolysis). Methods that assigned scores to different behavioral signs of estrus (like standing heat, sniffing, chin resting; for example the protocol used by Van Eerdenburg et al., 2002) can be used to model the intensity of estrous behavior. Heat scores associated with endocrine profiles around estrus could be used for parameter estimation. Taking the effects mentioned in this paragraph simultaneously into account in the model will likely generate new hypotheses on how the intensity of estrous behavior and the synchronization of estrous behavior and ovulation is regulated.

Most of our current understanding of genomic regulation of estrous behavior is obtained from studies in rodents (Chapter 2). However, in a recent study in cattle, where gene expression levels were measured at estrus and at mid-cycle, expression levels of several genes in the brain could be associated with intensity of estrous behavior (Kommadath et al., 2011). Genes associated with estrous behavior were e.g. oxytocin (*OXT*) and arginine vasopressin (*AVP*) (Kommadath et al., 2011). The identified genes could be related to processes of E2 and P4 dependent growth of behavior-directing hypothalamic neurons and to synchronization of estrous behavior with ovulation. Expression levels of these genes could be taken into account in the model by changing parameter configurations or by including novel model components to predict intensity of estrous behavior and to study interactions between genomic and endocrine effects on estrous cycle regulation.

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Appendices

1A. List of model equations[1] of Chapter 3. The equations listed below are the full notations of the equations developed in Section 3.3.2. Components numbering and initial values can be found in Appendix 1C.

Equation number	Equation
1.	$\frac{d}{dt} GnRH_{Hypo}(t) = Syn_{GnRH}(t) - Rel_{GnRH}(t)$
1a.	$Syn_{GnRH}(t) = c_{GnRH,1} \cdot (1 - \frac{GnRH_{Hypo}(t)}{GnRH_{Hypo}^{max}})$
1b.	$Rel_{GnRH}(t) = (H_1^-(P4\&E2) + H_2^-(P4)) \cdot GnRH_{Hypo}(t)$
2.	$\frac{d}{dt} GnRH_{Pit}(t) = Rel_{GnRH}(t) \cdot H_3^+(E2) - c_{GnRH,2} \cdot GnRH_{Pit}(t)$
3.	$\frac{d}{dt} FSH_{Pit}(t) = Syn_{FSH}(t) - Rel_{FSH}(t)$
3a.	$Syn_{FSH}(t) = H_4^-(Inh_\tau)$
3b.	$Rel_{FSH}(t) = (H_5^+(P4) + H_6^-(E2) + H_7^+(GnRH_{Pit})) \cdot FSH_{Pit}(t)$
4.	$\frac{d}{dt} FSH_{Blood}(t) = Rel_{FSH}(t) - c_{FSH} \cdot FSH_{Blood}(t)$
5.	$\frac{d}{dt} LH_{Pit}(t) = Syn_{LH}(t) - Rel_{LH}(t)$
5a.	$Syn_{LH}(t) = H_8^+(E2) + H_9^-(P4)$
5b.	$Rel_{LH}(t) = (b_{LH} + H_{10}^+(GnRH_{Pit})) \cdot LH_{Pit}(t)$
6.	$\frac{d}{dt} LH_{Blood}(t) = Rel_{LH}(t) - c_{LH} \cdot LH_{Blood}(t)$
7.	$\frac{d}{dt} Foll(t) = H_{11}^+(FSH_{blood}) - (H_{12}^+(P4) + H_{13}^+(LH_{blood})) \cdot Foll(t)$
8.	$\frac{d}{dt} PGF2\alpha(t) = H_{14}^+(P4_{\tau 1}) - H_{15}^+(P4_{\tau 2}) \cdot PGF2\alpha(t)$
9.	$\frac{d}{dt} CL(t) = H_{16}^+(LH_\tau) + H_{17}^+(CL) - H_{18}^+(PGF2\alpha) \cdot CL(t)$
10.	$\frac{d}{dt} P4(t) = c_{CL}^{P4} \cdot CL(t) - c_{P4} \cdot P4(t)$
11.	$\frac{d}{dt} E2(t) = c_{Foll}^{E2} \cdot Foll(t) - c_{E2} \cdot E2(t)$
12.	$\frac{d}{dt} Inh(t) = c_{Foll}^{Inh} \cdot Foll(t) - c_{Inh} \cdot Inh(t)$

[1]For abbreviation of the notation of Hill functions, we use *H(substrate)* instead of *m*h(substrate(t);T,n)*, where *n* is the steepness coefficient. H^+ = positive Hill function, H^- = negative Hill function, *T* = threshold for change of behavior of the Hill functions, and *m* = parameter that controls the height of the switch of the Hill functions. *Syn* = synthesis, *Rel* = release, *Pit* = pituitary, *Hypo* = hypothalamus, *c* = rate constant, τ = time delay, *Foll* = follicular function, *t* = time.

1B. List of Hill functions of Chapter 3. The Hill functions listed below are the full notations of the Hill functions mentioned in Section 3.3.2 and represent the mechanisms shown in Figure 3.2.

$H_1^-(P4\&E2) := m_{P4\&E2} \cdot \left(h^-\left(P4(t); T_{P4}^{GnRH,1}, 2\right) + h^-\left(E2(t), T_{E2}^{GnRH,1}, 2\right) - h^-\left(P4(t); T_{P4}^{GnRH,1}, 2\right) \cdot h^-\left(E2(t), T_{E2}^{GnRH,1}, 2\right)\right)$

$H_2^-(P4) := m_{P4}^{GnRH,2} \cdot h^-(P4(t), T_{P4}^{GnRH,2}, 2)$

$H_3^+(E2) := m_{E2}^{GnRH,2} \cdot h^+(E2(t), T_{E2}^{GnRH,2}, 5)$

$H_4^-(Inh_\tau) := m_{Inh}^{FSH} \cdot h^-(Inh(t - \tau_{Inh}), T_{Inh}^{FSH}, 5)$

$H_5^+(P4) := m_{P4}^{FSH} \cdot h^+(P4(t); T_{P4}^{FSH}, 2)$

$H_6^-(E2) := m_{E2}^{FSH} \cdot h^-(E2(t); T_{E2}^{FSH}, 2)$

$H_7^+(GnRH_{Pit}) := m_{GnRH}^{FSH} \cdot h^+(GnRH_{Pit}(t); T_{GnRH}^{FSH}, 1)$

$H_8^+(E2) := m_{E2}^{LH} \cdot h^+(E2(t); T_{E2}^{LH}, 2)$

$H_9^-(P4) := m_{P4}^{LH} \cdot h^-(P4(t); T_{P4}^{LH}, 2)$

$H_{10}^+(GnRH_{Pit}) := m_{GnRH}^{LH} \cdot h^+(GnRH_{Pit}(t); T_{GnRH}^{LH}, 5)$

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

$\tilde{T}_{FSH}^{Foll}(t) := T_{FSH}^{Foll} \cdot h^-(Foll(t); T_{Foll}^{FSH}, 2)$

$H_{12}^+(P4) := m_{P4}^{Foll} \cdot h^+(P4(t); T_{P4}^{Foll}, 2)$

$H_{13}^+(LH) := m_{LH}^{Foll} \cdot h^+(LH_{Blood}(t); T_{LH}^{Foll}, 2)$

$H_{14}^+(P4_\tau) := m_{P4}^{PGF2\alpha,1} \cdot h^+(P4\left(t - \tau_{P4,1}\right); T_{P4}^{PGF2\alpha}, 2)$

$H_{15}^+(P4_\tau) := m_{P4}^{PGF2\alpha,2} \cdot h^+(P4\left(t - \tau_{P4,2}\right); T_{P4}^{PGF2\alpha}, 10)$

$H_{16}^+(LH_\tau) := m_{LH}^{CL} \cdot h^+(LH_{blood}(t - \tau_{LH}); T_{LH}^{CL}, 2)$

$H_{17}^+(CL) := m_{CL}^{CL} \cdot h^+(CL(t); T_{CL}^{CL}, 2)$

$H_{18}^+(PGF2\alpha) := m_{PGF2\alpha}^{CL} \cdot h^+(PGF2\alpha(t); T_{PGF2\alpha}^{CL}, 1)$

1C. Initial values of the model described in Chapter 3.

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	$P4$	0.9995
11	$E2$	0.009995
12	Inh	0.1001

1D. List of parameters of Chapter 3. In our model, [.] stands for 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. t denotes 'time'; in our model [t] stands for 'days'.

No.	Parameter	Value	Dimension	Description
1	$c_{GnRH,1}$	4.657	$\frac{[GnRH_{Hypo}]}{[t]}$	Synthesis rate constant of GnRH in the hypothalamus
2	$GnRH_{Hypo}^{max}$	20.00	$[GnRH_{Hypo}]$	Maximum value for GnRH in the hypothalamus
3	$m_{P4\&E2}^{GnRH}$	1.464	1/[t]	Maximum part of GnRH synthesis rate constant inhibited by E2 and P4
4	$T_{E2}^{GnRH,1}$	0.1433	[E2]	Threshold of E2 to suppress GnRH release
5	$T_{P4}^{GnRH,1}$	0.0294	[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	[P4]	Threshold of P4 to inhibit GnRH release directly
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}^{FFSH}	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
19	T_{GnRH}^{FSH}	0.4	$[GnRH_{Pit}]$	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	$[GnRH_{Pit}]$	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
31	T_{Foll}^{FSH}	0.3	[Foll]	Threshold of follicular function to downscale FSH threshold
32	m_{P4}^{Foll}	2.5	1/[t]	Maximum decrease of follicular function stimulated by P4
33	T_{P4}^{Foll}	0.1127	[P4]	Threshold of P4 to stimulate decrease of follicular function
34	m_{LH}^{Foll}	2.8	1/[t]	Maximum decrease of follicular function stimulated by LH
35	T_{LH}^{Foll}	0.525	[LH]	Threshold of LH to stimulate decrease of follicular function
36	$\tau_{P4,1}$	12	[t]	Delay of P4 until stimulating PGF2α increase
37	$m_{P4}^{PGF2\alpha,1}$	0.3	[PGF2α]/[t]	Maximum growth rate of PGF2α
38	$T_{OT}^{PGF2\alpha,1}$	0.1672	[P4]	Threshold of P4 to stimulate PGF2α increase
39	$\tau_{P4,2}$	17	[t]	Delay of P4 until stimulating PGF2α decrease
40	$m_{P4}^{PGF2\alpha,2}$	11	[PGF2α]/[t]	Maximum decay rate of PGF2α
41	$T_{OT}^{PGF2\alpha,2}$	0.0966	[P4]	Threshold of P4 to stimulate PGF2α decrease
42	τ_{LH}	4.5	[t]	Delay of LH in CL
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 PGF2α
48	$T_{PGF2\alpha}^{CL}$	2	[PGF2α]	Threshold of PGF2α to initiate decrease of CL
49	c_{CL}^{P4}	3.856	$\frac{[P4]/[CL]}{[t]}$	Proportionality factor of CL in P4 increase
50	c_{P4}	2.737	1/[t]	P4 clearance rate constant
51	c_{Foll}^{E2}	1.9	$\frac{[E2]/[Foll]}{[t]}$	Proportionality factor of follicular function in E2 increase
52	c_{E2}	0.9	1/[t]	E2 clearance rate constant
53	c_{Foll}^{Inh}	4.8	$\frac{[Inh]/[Foll]}{[t]}$	Proportionality factor of delayed follicular function in Inh increase
54	c_{Inh}	4	1/[t]	Inh clearance rate constant

2A. List of model equations[1] of Chapter 4.

Equation number	Equation
1.	$\frac{d}{dt}GnRH_{Hypo}(t) = Syn_{GnRH}(t) - Rel_{GnRH}(t)$
1a.	$Syn_{GnRH}(t) = c_{GnRH,1} \cdot (1 - \frac{GnRH_{Hypo}(t)}{GnRH_{Hypo}^{max}})$
1b.	$Rel_{GnRH}(t) = (H_1^-(P4\&E2) + H_2^-(P4)) \cdot GnRH_{Hypo}(t)$
2.	$\frac{d}{dt}GnRH_{Pit}(t) = Rel_{GnRH}(t) \cdot H_3^+(E2) - c_{GnRH,2} \cdot GnRH_{Pit}(t)$
3.	$\frac{d}{dt}FSH_{Pit}(t) = Syn_{FSH}(t) - Rel_{FSH}(t)$
3a.	$Syn_{FSH}(t) = H_4^-(Inh_\tau)$
3b.	$Rel_{FSH}(t) = H_5^+(P4) + H_6^-(E2) + H_7^+(GnRH_{Pit})) \cdot FSH_{Pit}(t)$
4.	$\frac{d}{dt}FSH_{Blood}(t) = Rel_{FSH}(t) - c_{FSH} \cdot FSH_{Blood}(t)$
5.	$\frac{d}{dt}LH_{Pit}(t) = Syn_{LH}(t) - Rel_{LH}(t)$
5a.	$Syn_{LH}(t) = H_8^+(E2) + H_9^-(P4)$
5b.	$Rel_{LH}(t) = (b_{LH} + H_{10}^+(GnRH_{Pit})) \cdot LH_{Pit}(t)$
6.	$\frac{d}{dt}LH_{Blood}(t) = Rel_{LH}(t) - c_{LH} \cdot LH_{Blood}(t)$
7.	$\frac{d}{dt}Foll(t) = H_{11}^+(FSH_{blood}) - (H_{12}^+(P4) + H_{13}^+(LH_{blood})) \cdot Foll(t)$
8.	$\frac{d}{dt}PGF2\alpha(t) = H_{14}^+(OTR) - c_{PGF2\alpha} \cdot PGF2\alpha(t)$
9.	$\frac{d}{dt}OTR(t) = c_{P4}^{OTR} \cdot P4(t) - c_{OTR} \cdot OTR(t)$
10.	$\frac{d}{dt}CL(t) = H_{15}^+(LH_\tau) + H_{16}^+(CL) - H_{17}^+(PGF2\alpha) \cdot CL(t)$
11.	$\frac{d}{dt}P4(t) = c_{CL}^{P4} \cdot CL(t) - c_{P4} \cdot P4(t)$
12.	$\frac{d}{dt}E2(t) = c_{Foll}^{E2} \cdot Foll(t) - c_{E2} \cdot E2(t)$
13.	$\frac{d}{dt}Inh(t) = c_{Foll}^{Inh} \cdot Foll(t) - c_{Inh} \cdot Inh(t)$

[1]For abbreviation of the notation of Hill functions, we use *H(substrate)* instead of *m*h(substrate(t);T,n)*, where *n* is the steepness coefficient. H^+ = positive Hill function, H^- = negative Hill function, *T* = threshold for change of behavior of the Hill functions, and *m* = parameter that controls the height of the switch of the Hill functions. *Syn* = synthesis, *Rel* = release, *Pit* = pituitary, *Hypo* = hypothalamus, *c* = rate constant, τ = time delay, *t* = time.

2B. List of Hill functions[1] of Chapter 4.

$H_1^-(P4\&E2) := m_{P4\&E2} \cdot \left(h^-\left(P4(t); T_{P4}^{GnRH,1}, 2\right) + h^-\left(E2(t), T_{E2}^{GnRH,1}, 2\right) - h^-\left(P4(t); T_{P4}^{GnRH,1}, 2\right) \cdot h^-\left(E2(t), T_{E2}^{GnRH,1}, 2\right)\right)$

$H_2^-(P4) := m_{P4}^{GnRH,2} \cdot h^-(P4(t), T_{P4}^{GnRH,2}, 2)$

$H_3^+(E2) := m_{E2}^{GnRH,2} \cdot h^+(E2(t), T_{E2}^{GnRH,2}, 5)$

$H_4^-(Inh_\tau) := m_{Inh}^{FSH} \cdot h^-(Inh(t - \tau_{Inh}), T_{Inh}^{FSH}, 2)$

$H_5^+(P4) := m_{P4}^{FSH} \cdot h^+(P4(t); T_{P4}^{FSH}, 2)$

$H_6^-(E2) := m_{E2}^{FSH} \cdot h^-(E2(t); T_{E2}^{FSH}, 2)$

$H_7^+(GnRH_{Pit}) := m_{GnRH}^{FSH} \cdot h^+(GnRH_{Pit}(t); T_{GnRH}^{FSH}, 1)$

$H_8^+(E2) := m_{E2}^{LH} \cdot h^+(E2(t); T_{E2}^{LH}, 2)$

$H_9^-(P4) := m_{P4}^{LH} \cdot h^-(P4(t); T_{P4}^{LH}, 2)$

$H_{10}^+(GnRH_{Pit)} := m_{GnRH}^{LH} \cdot h^+(GnRH_{Pit}(t); T_{GnRH}^{LH}, 5)$

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

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

$H_{12}^+(P4) := m_{P4}^{Foll} \cdot h^+(P4(t); T_{P4}^{Foll}, 2)$

$H_{13}^+(LH) := m_{LH}^{Foll} \cdot h^+(LH_{Blood}(t); T_{LH}^{Foll}, 2)$

$H_{14}^+(OTR) := m_{OTR}^{PGF2\alpha} \cdot h^+(OTR(t); T_{OTR}^{PGF2\alpha}, 5)$

$H_{15}^+(LH_\tau) := m_{LH}^{CL} \cdot h^+(LH_{blood}(t - \tau_{LH}); T_{LH}^{CL}, 2)$

$H_{16}^+(CL) := m_{CL}^{CL} \cdot h^+(CL(t); T_{CL}^{CL}, 2)$

$H_{17}^+(PGF2\alpha) := m_{PGF2\alpha}^{CL} \cdot h^+(PGF2\alpha(t); T_{PGF2\alpha}^{CL}, 1)$

[1]See Table 4.1 and Appendix 2A for abbreviations.

2C. List of parameters and parameter values of the initial 3-wave cycle of Chapter 4

No.	Parameter[1]	Value	Dimension	Description[2]
1	$c_{GnRH,1}$	2.03	$\frac{[GnRH_{Hypo}]}{[t]}$	Synthesis rate constant of GnRH in the hypothalamus
2	$GnRH_{Hypo}^{max}$	20.00	$[GnRH_{Hypo}]$	Maximum value for GnRH in the hypothalamus
3	$m_{P4\&E2}^{GnRH}$	1.27	1/[t]	Maximum part of GnRH synthesis rate constant inhibited by E2 and P4
4	$T_{E2}^{GnRH,1}$	0.14	[E2]	Threshold of E2 to suppress GnRH release
5	$T_{P4}^{GnRH,1}$	0.03	[P4]	Threshold of P4 to allow E2 suppression of GnRH release
6	$m_{P4}^{GnRH,2}$	1.31	1/[t]	Maximum part of GnRH synthesis rate constant inhibited by P4
7	$T_{P4}^{GnRH,2}$	0.03	[P4]	Threshold of P4 to inhibit GnRH release directly
8	$m_{E2}^{GnRH,2}$	1.50	$\frac{[GnRH_{Pit}]}{[GnRH_{Hypo}]}$	Maximum scaling of pituitary sensitivity for GnRH
9	$T_{E2}^{GnRH,2}$	0.88	[E2]	Threshold of E2 to increase pituitary sensitivity for GnRH
10	$c_{GnRH,2}$	1.20	1/[t]	GnRH clearance rate constant in the pituitary
11	τ_{Inh}	1.41	[t]	Delay of Inh in FSH synthesis
12	m_{Inh}^{FFSH}	1.46	[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}	1.75	1/[t]	Maximum part of FSH release rate that is stimulated by P4
15	T_{P4}^{FSH}	0.10	[P4]	Threshold of P4 to stimulate FSH release
16	m_{E2}^{FSH}	0.26	1/[t]	Maximum part of FSH release rate that is inhibited by E2
17	T_{E2}^{FSH}	2.85	[E2]	Threshold of E2 to inhibit FSH release
18	m_{GnRH}^{FSH}	2.61	1/[t]	Maximum part of FSH release rate that is stimulated by GnRH
19	T_{GnRH}^{FSH}	0.20	$[GnRH_{Pit}]$	Threshold of GnRH to stimulate FSH release
20	c_{FSH}	0.78	1/[t]	FSH clearance rate constant
21	b_{FSH}	2.61	1/[t]	Basal FSH release
22	m_{E2}^{LH}	1.04	[LH]/[t]	Maximum part of LH synthesis that is stimulated by E2
23	T_{E2}^{LH}	0.10	[E2]	Threshold of E2 to stimulate LH synthesis
24	m_{P4}^{LH}	3.13	[LH]/[t]	Maximum part of LH synthesis that is inhibited by P4
25	T_{P4}^{LH}	0.03	[P4]	Threshold of P4 to inhibit LH synthesis
26	m_{GnRH}^{LH}	0.50	1/[t]	Maximum part of LH release rate that is stimulated by GnRH
27	T_{GnRH}^{LH}	0.50	$[GnRH_{Pit}]$	Threshold of GnRH to stimulate LH release
28	b_{LH}	0.04	1/[t]	basal LH release rate constant

29	c_{LH}	9.73	1/[t]	LH clearance rate constant
30	m_{FSH}^{Foll}	0.70	[Foll]/[t]	Maximum increase of follicular function stimulated by FSH
31	T_{FSH}^{Foll}	1.44	[FSH]	Threshold of FSH to stimulate follicular function
32	T_{Foll}^{FSH}	0.30	[Foll]	Threshold of follicular function to downscale FSH threshold
33	m_{P4}^{Foll}	2.17	1/[t]	Maximum decrease of follicular function stimulated by P4
34	T_{P4}^{Foll}	0.12	[P4]	Threshold of P4 to stimulate decrease of follicular function
35	m_{LH}^{Foll}	2.17	1/[t]	Maximum decrease of follicular function stimulated by LH
36	T_{LH}^{Foll}	0.42	[LH]	Threshold of LH to stimulate decrease of follicular function
37	$m_{OTR}^{PGF2\alpha}$	0.87	[OTR]/[t]	Maximum increase of PGF2α stimulated by OTR
38	$T_{OTR}^{PGF2\alpha}$	3.97	[OTR]	Threshold of OTR to stimulate PGF2α increase
39	$c_{PGF2\alpha}$	0.87	1/[t]	PGF2α clearance rate constant
40	τ_{LH}	4.52	[t]	Delay of LH in CL
41	m_{LH}^{CL}	0.29	[CL]/[t]	Maximum increase of CL stimulated by LH
42	T_{LH}^{CL}	0.96	[LH]	Threshold of LH to stimulate CL increase
43	m_{CL}^{CL}	0.06	[CL]/[t]	Maximum increase of CL stimulated by itself
44	T_{CL}^{CL}	0.07	[CL]	Threshold of CL to stimulate self-growth
45	$m_{PGF2\alpha}^{CL}$	5.69	1/[t]	Maximum decrease of CL stimulated by PGF2α
46	$T_{PGF2\alpha}^{CL}$	1.00	[PGF2α]	Threshold of PGF2α to initiate decrease of CL
47	c_{CL}^{P4}	1.45	$\frac{[P4]/[CL]}{1/[t]}$	Proportionality factor of CL in P4 increase
48	c_{P4}	0.58	1/[t]	P4 clearance rate constant
49	c_{P4}^{OTR}	0.87	$\frac{[OTR]/[CL]}{1/[t]}$	Maximum increase of OTR stimulated by P4
50	c_{OTR}	0.10	1/[t]	OTR decrease rate constant
51	c_{Foll}^{E2}	1.47	$\frac{[E2]/[Foll]}{[t]}$	Proportionality factor of follicular function in E2 increase
52	c_{E2}	0.96	1/[t]	E2 clearance rate constant
53	c_{Foll}^{Inh}	4.33	$\frac{[Inh]/[Foll]}{[t]}$	Proportionality factor of delayed follicular function in Inh increase
54	c_{Inh}	3.48	1/[t]	Inh clearance rate constant

[1]See Table 4.1 and Appendix 2A for definition of parameters.

3A. List of model equations[1] of Chapter 5

Equation number	Equation
1.	$\frac{d}{dt}GnRH_{Hypo}(t) = Syn_{GnRH}(t) - Rel_{GnRH}(t)$
1a.	$Syn_{GnRH}(t) = c_{GnRH,1} \cdot (1 - \frac{GnRH_{Hypo}(t)}{GnRH_{Hypo}^{max}})$
1b.	$Rel_{GnRH}(t) = (H_1^-(P4\&E2) + H_2^-(P4)) \cdot GnRH_{Hypo}(t)$
2.	$\frac{d}{dt}GnRH_{Pit}(t) = Rel_{GnRH}(t) \cdot H_3^+(E2) - c_{GnRH,2} \cdot GnRH_{Pit}(t)$
3.	$\frac{d}{dt}FSH_{Pit}(t) = Syn_{FSH}(t) - Rel_{FSH}(t)$
3a.	$Syn_{FSH}(t) = H_4^-(Inh)$
3b.	$Rel_{FSH}(t) = (b_{FSH} + H_5^+(P4) + H_6^-(E2) + H_7^+(GnRH_{Pit})) \cdot FSH_{Pit}(t)$
4.	$\frac{d}{dt}FSH_{Blood}(t) = Rel_{FSH}(t) - c_{FSH} \cdot FSH_{Blood}(t)$
5.	$\frac{d}{dt}LH_{Pit}(t) = Syn_{LH}(t) - Rel_{LH}(t)$
5a.	$Syn_{LH}(t) = H_8^+(E2) + H_9^-(P4)$
5b.	$Rel_{LH}(t) = (b_{LH} + H_{10}^+(GnRH_{Pit})) \cdot LH_{Pit}(t)$
6.	$\frac{d}{dt}LH_{Blood}(t) = Rel_{LH}(t) - c_{LH} \cdot LH_{Blood}(t)$
7.	$\frac{d}{dt}Foll(t) = H_{11}^+(FSH_{blood}) - (H_{12}^+(P4) + H_{13}^+(LH_{blood})) \cdot Foll(t)$
8.	$\frac{d}{dt}CL(t) = SF \cdot H_{13}^+(LH_{blood}) \cdot Foll(t) + H_{14}^+(CL) - H_{15}^+(IOF) \cdot CL(t)$
9.	$\frac{d}{dt}P4(t) = c_{CL}^{P4} \cdot CL(t)^2 - c_{P4} \cdot P4(t)$
10.	$\frac{d}{dt}E2(t) = c_{Foll}^{E2} \cdot Foll(t)^2 - c_{E2} \cdot E2(t)$
11.	$\frac{d}{dt}Inh(t) = c_{Foll}^{Inh} \cdot Foll(t)^2 - c_{Inh} \cdot Inh(t)$
12.	$\frac{d}{dt}OTR(t) = H_{16}^+(P4) - c_{OTR} \cdot OTR(t)$
13.	$\frac{d}{dt}OT(t) = H_{17}^+(E2) \cdot CL(t)^2 - c_{OT} \cdot OT(t)$
14.	$\frac{d}{dt}IOF(t) = H_{18}^+(PGF2\alpha\&CL) - c_{IOF} \cdot IOF(t)$
15.	$\frac{d}{dt}PGF2\alpha(t) = H_{19}^+(OTR\&OT) - c_{PGF2\alpha} \cdot PGF2\alpha(t)$

[1]For abbreviation of the notation of Hill functions, we use *H(substrate)* instead of *m*h(substrate(t);T,n)*, where H^+ = positive Hill function, H^- = negative Hill function, *m* = parameter that controls the height of the switch of the Hill functions, *t* = time, *T* = threshold for change of behavior of the Hill functions, and *n* is the steepness coefficient. *Syn* = synthesis, *Rel* = release, *Pit* = pituitary, *Hypo* = hypothalamus, *max* = maximum; *c* = rate constant.

3B. List of Hill functions[1] of Chapter 5.

$H_1^-\ (P4\&E2) := m_{P4\&E2} \cdot (h^-\left(P4(t); T_{P4}^{GnRH,1}, 2\right) + h^-\left(E2(t), T_{E2}^{GnRH,1}, 2\right) - h^-\left(P4(t); T_{P4}^{GnRH,1}, 2\right) \cdot h^-\left(E2(t), T_{E2}^{GnRH,1}, 2\right))$

$H_2^-(P4) := m_{P4}^{GnRH,2} \cdot h^-(P4(t), T_{P4}^{GnRH,2}, 2)$

$H_3^+(E2) := m_{E2}^{GnRH,2} \cdot h^+(E2(t), T_{E2}^{GnRH,2}, 5)$

$H_4^-(Inh) := m_{Inh}^{FSH} \cdot h^-(Inh(t), T_{Inh}^{FSH}, 5)$

$H_5^+(P4) := m_{P4}^{FSH} \cdot h^+(P4(t); T_{P4}^{FSH}, 2)$

$H_6^-(E2) := m_{E2}^{FSH} \cdot h^-(E2(t); T_{E2}^{FSH}, 2)$

$H_7^+(GnRH_{Pit}) := m_{GnRH}^{FSH} \cdot h^+(GnRH_{Pit}(t); T_{GnRH}^{FSH}, 1)$

$H_8^+(E2) := m_{E2}^{LH} \cdot h^+(E2(t); T_{E2}^{LH}, 2)$

$H_9^-(P4) := m_{P4}^{LH} \cdot h^-(P4(t); T_{P4}^{LH}, 2)$

$H_{10}^+(GnRH_{Pit)} := m_{GnRH}^{LH} \cdot h^+(GnRH_{Pit}(t); T_{GnRH}^{LH}, 5)$

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

$\tilde{T}_{FSH}^{Foll}(t) := T_{FSH}^{Foll} \cdot h^-(Foll(t); T_{Foll}^{FSH}, 2)$

$H_{12}^+(P4) := m_{P4}^{Foll} \cdot h^+(P4(t); T_{P4}^{Foll}, 5)$

$H_{13}^+(LH) := m_{LH}^{Ovul.\ Foll} \cdot h^+(LH_{Blood}(t); T_{LH}^{Ovul.\ Foll}, 2)$

$H_{14}^+(CL) := m_{CL}^{CL} \cdot h^+(CL(t); T_{CL}^{CL}, 2)$

$H_{15}^+(IOF) := m_{IOF}^{CL} \cdot h^+(IOF(t); T_{IOF}^{CL}, 5)$

$H_{16}^+(P4) := m_{P4}^{OTR} \cdot h^+(P4(t); T_{P4}^{OTR}, 5)$

$H_{17}^+(E2) := m_{E2}^{OT} \cdot h^+(E2(t); T_{E2}^{OT}, 2)$

$H_{18}^+(PGF2\alpha\&CL) := m_{PGF2\alpha\&CL}^{IOF} \cdot h^+(PGF2\alpha(t); T_{PGF2\alpha}^{IOF}, 5) \cdot h^+(CL(t); T_{CL}^{IOF}, n_{CL}^{IOF}, 10)$

$H_{19}^+(OTR\&OT) := m_{OTR\&OT}^{PGF2\alpha} \cdot h^+(OTR(t); T_{OTR}^{PGF2\alpha}, 5) \cdot h^+(OT(t); T_{OT}^{PGF2\alpha}, 2)$

[1]In the differential equations, Hill functions were used for the modeling of inhibitory and stimulatory effects of hormones. A Hill function is a sigmoidal function between 0 and 1, which switches at a specified threshold from one level to the other with a specified steepness. The notation of the Hill functions is *m*h(substrate(t);T,n)*, where H^+ = positive Hill function, H^- = negative Hill function, and *m* = parameter that controls the height of the switch of the Hill functions, *t* = time, *T* = threshold for change of behavior of the Hill functions, and *n* is the steepness coefficient.

3C. List of parameters and parameter values of the initial 3-wave cycle of Chapter 5.

No.	Parameter[1]	Value	Dimension	Description
1	$c_{GnRH,1}$	2.75	$\frac{[GnRH_{Hypo}]}{[t]}$	Synthesis rate constant of GnRH in the hypothalamus
2	$GnRH_{Hypo}^{max}$	16.00	$[GnRH_{Hypo}]$	Maximum value for GnRH in the hypothalamus
3	$m_{P4\&E2}^{GnRH}$	2.05	1/[t]	Maximum part of GnRH synthesis rate constant inhibited by E2 and P4
4	$T_{E2}^{GnRH,1}$	0.10	[E2]	Threshold of E2 to suppress GnRH release
5	$T_{P4}^{GnRH,1}$	0.35	[P4]	Threshold of P4 to allow E2 suppression of GnRH release
6	$m_{P4}^{GnRH,2}$	1.91	1/[t]	Maximum part of GnRH synthesis rate constant inhibited by P4
7	$T_{P4}^{GnRH,2}$	0.25	[P4]	Threshold of P4 to inhibit GnRH release directly
8	$m_{E2}^{GnRH,2}$	0.99	$\frac{[GnRH_{Pit}]}{[GnRH_{Hypo}]}$	Maximum scaling of pituitary sensitivity for GnRH
9	$T_{E2}^{GnRH,2}$	0.65	[E2]	Threshold of E2 to increase pituitary sensitivity for GnRH
10	$c_{GnRH,2}$	1.63	1/[t]	GnRH clearance rate constant in the pituitary
11	m_{Inh}^{FFSH}	4.21	[FSH]/[t]	Maximum FSH synthesis rate in the pituitary in the absence of Inh
12	T_{Inh}^{FSH}	0.12	[Inh]	Threshold of Inh for inhibition of FSH synthesis
13	m_{P4}^{FSH}	0.29	1/[t]	Maximum part of FSH release rate that is stimulated by P4
14	T_{P4}^{FSH}	0.15	[P4]	Threshold of P4 to stimulate FSH release
15	m_{E2}^{FSH}	0.40	1/[t]	Maximum part of FSH release rate that is inhibited by E2
16	T_{E2}^{FSH}	0.31	[E2]	Threshold of E2 to inhibit FSH release
17	m_{GnRH}^{FSH}	1.23	1/[t]	Maximum part of FSH release rate that is stimulated by GnRH
18	T_{GnRH}^{FSH}	0.07	$[GnRH_{Pit}]$	Threshold of GnRH to stimulate FSH release
19	c_{FSH}	2.73	1/[t]	FSH clearance rate constant
20	b_{FSH}	0.95	1/[t]	Basal FSH release
21	m_{E2}^{LH}	0.38	[LH]/[t]	Maximum part of LH synthesis that is stimulated by E2
22	T_{E2}^{LH}	0.24	[E2]	Threshold of E2 to stimulate LH synthesis
23	m_{P4}^{LH}	2.71	[LH]/[t]	Maximum part of LH synthesis that is inhibited by P4
24	T_{P4}^{LH}	0.03	[P4]	Threshold of P4 to inhibit LH synthesis
25	m_{GnRH}^{LH}	2.22	1/[t]	Maximum part of LH release rate that is stimulated by GnRH
26	T_{GnRH}^{LH}	0.69	$[GnRH_{Pit}]$	Threshold of GnRH to stimulate LH release
27	b_{LH}	0.01	1/[t]	basal LH release rate constant
28	c_{LH}	12.0	1/[t]	LH clearance rate constant

29	m_{FSH}^{Foll}	0.56	[Foll]/[t]	Maximum increase of follicular function stimulated by FSH
30	T_{FSH}^{Foll}	0.57	[FSH]	Threshold of FSH to stimulate follicular function
31	T_{Foll}^{FSH}	0.22	[Foll]	Threshold of follicular function to downscale FSH threshold
32	m_{P4}^{Foll}	1.10	1/[t]	Maximum decrease of follicular function stimulated by P4
33	T_{P4}^{Foll}	0.13	[P4]	Threshold of P4 to stimulate decrease of follicular function
34	$m_{LH}^{Ovul.\ Foll}$	3.49	1/[t]	Maximum decrease of follicular function stimulated by LH
35	$T_{LH}^{Ovul.\ Foll}$	0.17	[LH]	Threshold of LH to stimulate decrease of follicular function
36	$m_{OTR\&OT}^{PGF2\alpha}$	53.91	[PGF2α]/[t]	Maximum increase of PGF2α stimulated by OTR and OT
37	$T_{OTR}^{PGF2\alpha}$	1.43	[OTR]	Threshold of OTR to stimulate PGF2α increase
38	$T_{OT}^{PGF2\alpha}$	1.09	[OT]	Threshold of OT to stimulate PGF2α increase
39	$c_{PGF2\alpha}$	1.23	1/[t]	PGF2α clearance rate constant
40	SF	0.20	[CL]/[t]	Scaling factor of CL
41	m_{CL}^{CL}	0.04	[CL]/[t]	Maximum increase of CL stimulated by itself
42	T_{CL}^{CL}	0.10	[CL]	Threshold of CL to stimulate self-growth
43	m_{IOF}^{CL}	41.39	1/[t]	Maximum decrease of CL stimulated by IOF
44	T_{IOF}^{CL}	1.32	[IOF]	Threshold of IOF to stimulate CL decrease
45	c_{CL}^{P4}	2.25	$\frac{[P4]/[CL]^2}{[t]}$	Proportionality factor of CL in P4 increase
46	c_{P4}	1.41	1/[t]	P4 clearance rate constant
47	c_{Foll}^{E2}	2.19	$\frac{[E2]/[Foll]^2}{[t]}$	Proportionality factor of follicular function in E2 increase
48	c_{E2}	1.23	1/[t]	E2 clearance rate constant
49	c_{Foll}^{Inh}	1.41	$\frac{[Inh]/[Foll]^2}{[t]}$	Proportionality factor of delayed follicular function in Inh increase
50	c_{Inh}	0.48	1/[t]	Inh clearance rate constant
51	m_{P4}^{OTR}	3.58	[OTR]/[t]	Maximum increase of OTR stimulated by P4
52	T_{P4}^{OTR}	0.77	[P4]	Threshold of P4 to stimulate OTR increase
53	c_{OTR}	2.98	1/[t]	OTR decrease rate constant
54	m_{E2}^{OT}	1.59	$\frac{[OT]/[CL]^2}{[t]}$	Maximum increase of OT stimulated by E2
55	T_{E2}^{OT}	0.14	[E2]	Threshold of E2 to stimulate OT increase
56	c_{OT}	0.64	1/[t]	OT decrease rate constant
57	$m_{PGF2\alpha\&CL}^{IOF}$	39.68	[IOF]/[t]	Maximum increase of IOF stimulated by PGF2α and CL
58	$T_{PGF2\alpha}^{IOF}$	1.22	[PGF2α]	Threshold of PGF2α to stimulate IOF increase
59	T_{CL}^{IOF}	0.60	[CL]	Threshold of CL to stimulate IOF increase
60	c_{IOF}	0.30	1/[t]	IOF decrease rate constant

[1]*max* = maximum, *m* = parameter that controls the height of the switch of the Hill functions, *T* = threshold for change of behavior of the Hill functions, *b* = basal rate constant, *c* = clearance rate constant, *Ovul.Foll* = ovulatory follicle, *Hypo* = hypothalamus, *t* = time, *Pit* = pituitary.

English summary

Systems biology is a relatively new research area in the field of reproduction physiology. 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. Such a mathematical model delivers 1) quantification of known biological processes, 2) model predictions for various conditions, and 3) new hypotheses and predictions about regulation of the system under study. One example of a dynamic biological system is the bovine estrous cycle, which consists of hormonally controlled recurrent periods when the cow is preparing for reproduction by producing a fertilizable oocyte. Bovine fertility is the subject of extensive research in animal sciences, especially since, coinciding with selection for increased milk yield, a decrease in dairy cow fertility has been observed during the last decades. This decline in fertility has been observed as decreased conception rates, prolonged calving intervals, reduced heat detection due to reduced expression of estrous behavior as well as alterations in hormone patterns during the estrous cycle. However, it is difficult to understand which underlying mechanisms cause this decline in fertility. The regulation of estrus is controlled by the interplay of various organs and hormones. Mathematical modeling of the involved mechanisms is expected to gain more insight in the biological processes underlying the bovine estrous cycle, and could thereby help to find the causes of declined fertility in dairy cows. The objective of this thesis was to improve insight in the regulation of dairy cow fertility by developing and using a mechanistic mathematical model of the bovine estrous cycle.

In dairy cows, the optimal time of artificial insemination is signaled by estrous behavior. Selection for milk yield has coincided with a decline in duration and intensity of estrus, making heat detection and determining the optimal moment of insemination more difficult. The endocrine regulation of the estrous cycle in cows is well described, but a clear understanding of how this is related to estrous behavior is lacking. Chapter 2 of this thesis is a literature review that describes current knowledge about (genomic regulation of) expression of estrous behavior. This chapter describes the key role that E2 plays in regulation of the estrous cycle and estrous behavior and also how E2 interacts with P4 and many other factors that together regulate the cycle. Although endocrine regulation of the estrous cycle in general is extensively studied, till now relatively little research has been carried out on the control of estrous behavior as such. Most of our current

understanding of genomic regulation of estrous behavior is obtained from studies in rodents. These studies show that estrogen-induced gene expression in specific brain areas such as the hypothalamus alter the functioning of neuronal networks that underlie estrous behavior. It is important to have a concise overview of the dynamics of follicle and CL development and the involved endocrine interactions to understand how the expression of estrous behavior is related to the endocrine regulation of the cycle.

In Chapter 3 we describe the development of a mathematical model of the bovine estrous cycle, including the processes of follicle and CL development and the key hormones that interact to control these processes. Although the endocrine and physiologic regulation of the bovine estrous cycle is studied extensively, mathematical models of cycle regulation are scarce and of limited scope. The model we developed describes the dynamics of the bovine estrous cycle on individual cow level. It 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 and constructed a flow chart of their interactions. The flow chart was translated into a set of equations and parameters that describes the system consistent with biological data for cows. The parameters in the model represent kinetic properties of the system with regard to synthesis, release, and clearance of hormones as well as growth and regression of follicles and corpora lutea. Model parameters were estimated by comparing model simulations to known physiological behaviors and experimental data of relevant endocrine and physiological factors retrieved from literature. Since the literature review in Chapter 2 showed the critical role of E2 and P4 in the regulation of behavior, these hormones are also important output parameters in the development of the mathematical model. Even though the majority of the mechanisms in the model are based on qualitative relations described in literature (i.e. stimulation and inhibition), the output of the model is surprisingly well in line with empirical data. The model captures a number of key physiological processes of the bovine estrous cycle, and serves as a starting point for further simulation studies, model validation, and extended models.

In Chapter 4 we investigated which mechanisms could be candidates for regulation of the number of waves in the bovine estrous cycle. A normal bovine estrous cycle contains 2 or 3 waves in which a cohort of follicles starts to grow. Ovulation takes place in the last wave. The biological mechanisms that determine whether a cycle has 2 or 3 waves have not been elucidated. The model was initially parameterized to generate 3 waves of follicle development per cycle. We

hypothesized that some of the parameters related to follicle growth rate or to the time point of CL regression are candidates to affect the number of waves per cycle. We performed simulations with the model in which we varied the values of these parameters. It was shown that variation of (combinations of) model parameters regulating follicle growth rate or time point of CL regression can change the model output from 3 to 2 waves of follicular growth in a cycle. In addition, alternating 2- and 3-wave cycles occurred. Some of the parameter changes seem to represent plausible biological mechanisms that could explain these follicular wave patterns. It was for instance found that the model output changed from 3 to 2 waves in a cycle when the duration of the luteal phase was changed or when the effect of FSH or P4 on follicle growth was changed, but not when FSH production or P4 production was changed. Simulation results regarding repeatability of wave pattern, differences in cycle length, and differences in diameter of the dominant follicle between cycles with different number of waves were in line with literature. Hence, our simulations indicated candidates involved in the mechanisms that regulate the follicular wave pattern, increasing our understanding of the regulation of the estrous cycle in dairy cows.

In Chapter 5 we analyzed which parameter changes can lead to development of cystic ovaries, a common cause of reproductive failure in dairy cows. Several factors may cause perturbations in the regular oscillatory behavior of a normal estrous cycle. Such perturbations are likely the effect of simultaneous changes in multiple parameters. We tested how far the parameter values of the model could be disturbed without changing the characteristics of a normal cycle. In addition, we searched for parameter configurations simulating P4 profiles associated with cystic ovaries, i.e. delayed ovulation and delayed luteolysis. The results indicated that CL functioning, luteolytic signals, and GnRH synthesis are likely involved in the development of cystic ovaries. These findings could be investigated in future experiments. Further, the results indicated that delayed ovulation and delayed luteolysis could have the same cause and are characterized by irregular hormone patterns. An interesting pattern observed in the model simulations was the transition from one parameter configuration for a normal cycle to another, with the occurrence of both delayed ovulation and delayed luteolysis in parameter configurations in between. The method of parameter perturbation described in Chapter 5 is an effective tool to find parameter configurations that lead to P4 profiles associated with delayed ovulation and delayed luteolysis.

In Chapter 6 we fitted a number of model parameters to measurements of 31 normally cycling cows that show significant differences in for example peak levels and time courses of hormone profiles. These 31 cows with synchronized

estrous cycles provided the *in vivo* measurements on follicle and CL sizes, and P4, E2, FSH, and LH concentrations in plasma. Model parameter values were fitted to match the given *in vivo* data using a least squares optimization procedure. Finding specific parameter configurations for individual cows shows the capability of the model to simulate 'real' data. Thereby it was explored which parameter values varied between cows with different estrous cycle characteristics, e.g. follicle wave number and P4 levels. Certain combinations of estimated parameter values induce a clear qualitative shift in model behavior (e.g. a different number of follicular waves), suggesting possible routes how environmental or genetic influences could affect estrous cycle characteristics.

In Chapter 7 the modeling approach and the insights about dairy cow fertility that were obtained by developing and using the model of the bovine estrous cycle are discussed. The results of the previous chapters are combined and discussed from a model-based and a biology-based perspective. From the model-based perspective, it is discussed how the model can be further validated and improved. From the biology-based perspective, hypotheses are generated about critical points in the network of physiological events that determine estrous cycle dynamics and could have a relatively strong impact on fertility. For example, model simulations indicate that parameters involved in PGF2α induced luteolysis have a large effect on dynamics of the estrous cycle, and it is speculated how these hypotheses could be investigated in animal experiments. Finally, it is discussed how the model could be extended with effects of energy metabolism and estrous behavior.

Nederlandse samenvatting

Systeembiologie is een relatief nieuw onderzoeksterrein binnen de voortplantingsbiologie. Het heeft als doel om te begrijpen hoe de diverse componenten van een biologisch systeem tezamen functioneren, in plaats van alleen de individuele onderdelen te bestuderen. Een voorbeeld van systeembiologie is het omzetten van biologische kennis over een bepaald biologisch systeem naar een set van wiskundige vergelijkingen dat de interacties tussen componenten van dat systeem laat zien. Een dergelijk wiskundig model verschaft 1) kwantificatie van bekende biologische processen, 2) voorspellingen van het model voor diverse omstandigheden, en 3) nieuwe voorspellingen en hypotheses over de regulatie van het systeem dat wordt bestudeerd. Een voorbeeld van een biologisch systeem is de bronstcyclus van de koe. De bronstcyclus is de zich herhalende periode aangestuurd door hormonen, waarin de koe zich voorbereid om zich voort te planten door een vruchtbare eicel te produceren. Vruchtbaarheid van koeien wordt uitgebreid bestudeerd binnen de dierwetenschappen. Tegelijk met de selectie voor hoge melkgift is de vruchtbaarheid van melkkoeien namelijk achteruit gegaan tijdens de afgelopen decennia. Deze dalende vruchtbaarheid uit zich in een lager bevruchtingspercentage, langere tussenkalftijd, minder goede detectie van tocht (bronst) vanwege het minder duidelijk tonen van tochtgedrag (bronstgedrag) en afwijkende concentraties van hormonen in het bloed tijdens de bronstcyclus. Het is echter moeilijk om er achter te komen welke onderliggende mechanismen deze daling in vruchtbaarheid veroorzaken. De bronstcyclus wordt gereguleerd door de interacties tussen diverse organen en hormonen. Het wiskundig modelleren van de betrokken mechanismen kan meer inzicht opleveren in de biologische processen die de bronstcyclus reguleren. Het modelleren draagt daardoor bij aan het vinden van oorzaken van verminderde vruchtbaarheid in melkkoeien. Het doel van dit proefschrift was om meer inzicht te krijgen in de regulatie van vruchtbaarheid van melkkoeien via het ontwikkelen en gebruiken van een wiskundig model van de bronstcyclus van de koe.

Bij melkkoeien wordt het optimale tijdstip voor kunstmatige inseminatie bepaald door het laten zien van tochtgedrag (bronstgedrag). De genetische selectie voor hoge melkgift is samengegaan met een teruggang in de duur en intensiteit van tochtgedrag. Daardoor is het detecteren van tochtigheid (bronst) en het bepalen van het optimale moment van inseminatie moeilijker. De hormonale regulatie van de bronstcyclus van de koe is uitgebreid beschreven, maar er is nog veel onduidelijk over hoe dit is gerelateerd aan tochtgedrag. Hoofdstuk 2 van dit proefschrift is een literatuurstudie die de huidige kennis beschrijft op het gebied

van tochtgedrag en door welke genen dit gedrag wordt aangestuurd. Dit hoofdstuk beschrijft de belangrijke rol van oestradiol in de regulatie van de bronstcyclus en de interactie van oestradiol en progesteron met andere factoren die tezamen de cyclus aansturen. Hoewel de hormonale regulatie van de bronstcyclus tot in detail is bestudeerd, is er relatief weinig onderzoek gedaan op het gebied van de regulatie van tochtgedrag. Het merendeel van wat bekend is op het gebied van genen die tochtgedrag aansturen is ontleend aan onderzoeken bij knaagdieren (ratten en muizen). Die onderzoeken laten zien dat oestradiol genen aanstuurt in bepaalde hersengebieden, zoals de hypothalamus. Die genen sturen vervolgens seksueel gedrag aan. Om te begrijpen hoe het laten zien van tochtgedrag samenhangt met de hormonale regulatie van de bronstcyclus is het echter noodzakelijk om goed te begrijpen hoe verschillende hormonen die de ontwikkeling van follikels en het corpus luteum regelen met elkaar samenwerken. De rest van dit proefschrift gaat daarom over de regulatie van de bronstcyclus.

In Hoofdstuk 3 wordt de ontwikkeling beschreven van een wiskundig model van de bronstcyclus van de koe, met daarin de processen van follikel en corpus luteum ontwikkeling en de belangrijkste hormonen die tezamen deze processen aansturen. De hormonale en fysiologische regulatie van de bronstcyclus van de koe is uitgebreid bestudeerd, maar wiskundige modellen van de cyclus zijn schaars en beperkt van omvang. Het model dat wij hebben ontwikkeld, beschrijft de bronstcyclus op het niveau van de individuele koe. Het model is in staat om de ontwikkeling van follikels en het corpus luteum en de periodieke veranderingen in hormoonspiegels die deze ontwikkeling reguleren te simuleren met een set van differentiaalvergelijkingen die aan elkaar gekoppeld zijn. Eerst is er een literatuurstudie gedaan over hoe de individuele componenten van de cyclus (bijvoorbeeld de hormonen oestradiol en progesteron) tezamen functioneren. Op basis daarvan is een stroomdiagram gemaakt van de interacties van deze componenten. Het stroomdiagram is omgezet in een set van vergelijkingen en parameters dat de bronstcyclus van koeien nabootst in overeenstemming met biologische kennis. De parameters in het model representeren eigenschappen van de cyclus, zoals aanmaak, afgifte en afbraak van hormonen en groei en afbraak van follikels en het corpus luteum. Waardes voor parameters zijn geschat door modelsimulaties te vergelijken met literatuur over het verloop van hormoonconcentraties tijdens de cyclus. Omdat het literatuuronderzoek in Hoofdstuk 2 liet zien dat oestradiol en progesteron een hele belangrijke rol spelen bij tochtgedrag zijn deze twee hormonen ook belangrijke uitleesparameters in de ontwikkeling van het wiskundige model. De meeste mechanismen van het model zijn gebaseerd op interacties die alleen kwalitatief en niet kwantitatief beschreven

zijn in de literatuur (zoals stimuleren en afremmen), maar toch is de uitkomst van het model opmerkelijk goed in overeenstemming met experimentele metingen. Het model beschrijft de belangrijkste biologische processen van de bronstcyclus van de koe en dient als startpunt voor verdere simulatiestudies, validatie van het model en uitgebreidere modellen.

In Hoofdstuk 4 hebben we onderzocht welke mechanismen mogelijk het aantal folliculaire groeigolven reguleren in de bronstcyclus van de koe. Een normale cyclus bevat 2 of 3 groeigolven waarin een aantal follikels begint te groeien, waarbij ovulatie van de eicel plaatsvindt in de laatste groeigolf. De biologische mechanismen die bepalen of een cyclus 2 dan wel 3 groeigolven heeft, zijn echter niet duidelijk. De parameters van het model waren in eerste instantie zo gekozen dat het model 3 folliculaire groeigolven per cyclus simuleert. Onze hypothese was dat bepaalde parameters gerelateerd aan de groeisnelheid van follikels of aan het tijdstip waarop afbraak van het corpus luteum begint het aantal groeigolven in de cyclus kunnen beïnvloeden. We hebben simulaties uitgevoerd met het model waarin we de waardes van die parameters varieerden. De simulaties toonden aan dat variatie in (combinaties van) parameterwaardes die folliculaire groeisnelheid of tijdstip van corpus luteum afbraak reguleren de modeluitkomst kunnen veranderen van 3 naar 2 folliculaire groeigolven in een cyclus. Bovendien kwamen patronen voor waarin cycli met 2 en 3 groeigolven elkaar afwisselden. Bepaalde parameterveranderingen lijken een plausibele biologische verklaring te geven voor deze patronen van folliculaire groeigolven. De uitkomst van het model veranderde bijvoorbeeld van 3 naar 2 groeigolven wanneer de lengte van de luteale fase werd gevarieerd of wanneer het effect van FSH of progesteron op folliculaire groei werd gevarieerd. Simulatieresultaten waren in overeenstemming met de literatuur, zoals de herhaalbaarheid van het aantal groeigolven in opeenvolgende cycli, verschillen in de lengte van de cyclus en verschillen in de grootte van het dominante follikel tussen cycli met een verschil in het aantal groeigolven. Onze simulaties laten dus een aantal mogelijke mechanismen zien die het aantal folliculaire groeigolven reguleren, en vergroten daarmee ons inzicht in de regulatie van de bronstcyclus in melkkoeien.

In Hoofdstuk 5 hebben we onderzocht welke parameterveranderingen leiden tot de ontwikkeling van cysteuze ovaria, een veelvoorkomende afwijking die vruchtbaarheidsproblemen bij melkkoeien veroorzaakt. Bij deze afwijking vindt de afbraak van het follikel of van het corpus luteum later plaats dan normaal en dit is te zien aan afwijkende progesteronconcentraties in het bloed. Het gevolg hiervan is afwezigheid van tocht en verlengde cycli. Verscheidene factoren kunnen het normale verloop van de cyclus verstoren. Zulke verstoringen zijn waarschijnlijk het

effect van veranderingen in een aantal parameters tegelijkertijd. We hebben getest hoe ver de parameterwaardes van het model konden worden veranderd zonder dat het karakteristieke gedrag van een normale cyclus verandert. Daarnaast zochten we naar parameterconfiguraties (combinaties van parameterwaardes) die progesteronprofielen simuleren die geassocieerd zijn met cysteuze ovaria, namelijk verlate ovulatie en verlate luteolyse (afbraak van het corpus luteum). De resultaten suggereren dat signalen die de afbraak van het corpus luteum in gang zetten en aanmaak van het hormoon GnRH een rol spelen in de ontwikkeling van cysteuze ovaria. Deze bevindingen kunnen worden onderzocht in toekomstige experimenten. Bovendien laten de resultaten zien dat verlate ovulatie en verlate luteolyse dezelfde oorzaak zouden kunnen hebben en zijn gekarakteriseerd door onregelmatige hormoonpatronen. Een interessant patroon dat geobserveerd is in de modelsimulaties was de overgang van de ene parameterconfiguratie voor een normale cyclus naar een andere, waarbij zowel verlate ovulatie als verlate luteolyse optraden in tussenliggende parameterconfiguraties. De methode van parameterverandering zoals beschreven in Hoofdstuk 5 is een effectieve manier om parameterconfiguraties te vinden die leiden tot progesteronprofielen die geassocieerd zijn met verlate ovulatie en verlate luteolyse.

In Hoofdstuk 6 hebben we onderzocht hoe goed het model kan omgaan met verschillen tussen koeien in bijvoorbeeld piekniveaus en periodieke veranderingen in hormoonconcentraties in parameterwaardes. Daartoe werden data gebruikt van 31 koeien die een normale cyclus hadden, maar significante verschillen in bijvoorbeeld piekniveaus en periodieke veranderingen in hormoonconcentraties. Van deze 31 koeien waren metingen gedaan van follikel en corpus luteum afmetingen en van progesteron, oestradiol, FSH en LH concentraties in het bloed. Modelparameters werden met behulp van de zogenaamde kleinste kwadraten methode gekozen om zo optimaal mogelijk in overeenstemming te zijn met de metingen. Het vinden van specifieke parameterconfiguraties voor individuele koeien laat zien dat het model kan simuleren met ‘echte’ metingen. Daarmee kon worden onderzocht welke parameterwaardes variëren tussen koeien met verschillen in de cyclus, zoals het aantal folliculaire groeigolven en progesteronconcentraties. Bepaalde configuraties van geschatte parameterwaardes leidden tot een duidelijke kwalitatieve verandering in het gedrag van het model (bijvoorbeeld een ander aantal folliculaire groeigolven), wat mogelijke routes suggereert waarlangs omgevings- of genetische invloeden de cyclus kunnen beïnvloeden.

In Hoofdstuk 7 worden de manier van modelleren en de inzichten over vruchtbaarheid van melkkoeien die zijn verkregen door het ontwikkelen en

gebruiken van het model van de bronstcyclus van de koe bediscussieerd. De resultaten van de voorgaande hoofdstukken worden gecombineerd en besproken vanuit een modelmatig en een biologisch perspectief. Vanuit modelmatig perspectief wordt besproken hoe het model verder kan worden gevalideerd en verbeterd. Vanuit biologisch perspectief worden hypotheses gegenereerd over kritieke punten in het netwerk van fysiologische gebeurtenissen die de periodieke veranderingen tijdens de bronstcyclus bepalen en een relatief sterke invloed op vruchtbaarheid kunnen hebben. Simulaties met het model suggereren bijvoorbeeld dat parameters betrokken bij afbraak van het corpus luteum door het hormoon PGF2α een groot effect hebben op het hele verloop van de bronstcyclus. Er is gekeken hoe dergelijke hypotheses onderzocht zouden kunnen worden in dierexperimenten. Tenslotte wordt besproken hoe het model kan worden uitgebreid met effecten van energiemetabolisme en tochtgedrag om daarmee meer inzicht te krijgen in de verminderde vruchtbaarheid en tochtgedrag bij melkkoeien.

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Curriculum Vitae

Hendrika Martha Theodora (Marike) Boer werd op 24 april 1984 geboren in Huizen. Vanaf haar zesde groeide zij op in Putten. In 2002 behaalde zij haar VWO diploma aan het Johannes Fontanus College in Barneveld. Daarna begon zij in Wageningen aan de Bachelor opleiding Dierwetenschappen. Ze heeft zich tijdens haar Master gespecialiseerd in de richting Fokkerij en Genetica. Tijdens haar Master heeft zij een stage gedaan bij IPG (Institute for Pig Genetics) met als onderwerp voeropname van zeugen tijdens de lactatie. Haar afstudeerscriptie ging over effecten van inteelt op spermakwaliteit van Friese hengsten. Na het behalen van haar Master begon zij in 2008 als AIO bij de vakgroep Adaptatiefysiologie van Wageningen Universiteit, in samenwerking met het Animal Breeding and Genomics Centre van Wageningen UR Livestock Research. De resultaten van dit onderzoek zijn beschreven in dit proefschrift.

Publications in scientific journals

- H.M.T. Boer, M. Apri, J. Molenaar, C. Stötzel, R.F. Veerkamp, H. Woelders. 2012. Candidate mechanisms underlying atypical progesterone profiles as deduced from parameter perturbations in a mathematical model of the bovine estrous cycle. Journal of Dairy Science 95:3837-3851.
- H.M.T. Boer, S. Röblitz, C. Stötzel, R.F. Veerkamp, B. Kemp, H. Woelders. 2011. Mechanisms regulating follicle wave patterns in the bovine estrous cycle investigated with a mathematical model. Journal of Dairy Science 94:5987-6000.
- H.M.T. Boer, C. Stötzel, S. Röblitz, P. Deuflhard, R.F. Veerkamp, H. Woelders. 2011. A simple mathematical model of the bovine estrous cycle: follicle development and endocrine interactions. Journal of Theoretical Biology 278(1):20-31.
- H.M.T. Boer, R.F. Veerkamp, B. Beerda, H. Woelders. 2010. Estrous behavior in dairy cows: identification of underlying mechanisms and gene functions. Animal 4:446-453.

Book chapters

- H.M.T. Boer, C. Stötzel, S. Röblitz, H. Woelders. 2012. A differential equation model to investigate the dynamics of the bovine estrous cycle. In: Advances in Experimental Medicine and Biology, Volume 736, p597-605, Springer, New York.

Conference proceedings

- S.L. Shields, H. Woelders, H.M.T. Boer, S. Röblitz, C Stötzel, J. Plöntzke, J.P. McNamara. Integrating nutritional and reproductive models to improve reproductive efficiency in dairy cattle. Journal of Dairy Science 95(Suppl. 2):46, Joint annual meeting abstracts of ADSA-ASAS Joint Annual Meeting, Phoenix, Arizona, 15-19 July 2012.
- C. Stötzel, H.M.T. Boer, J. Plöntzke, S. Röblitz. Applications of a mathematical model for the bovine estrous cycle. In: Abstract book of the 12th International Conference on Systems Biology (ICSB) PS230, Heidelberg, Germany, 28 August - 1 September 2011.
- H.M.T. Boer, S. Röblitz, C. Stötzel, R.F. Veerkamp, B. Kemp, H. Woelders. Two- and three-wave estrous cycles in dairy cows, investigated with a mechanistic mathematical model. In: *Journal of Dairy Science* 94, E-Supplement 1, Joint annual meeting abstracts of ADSA-ASAS Joint Annual Meeting, New Orleans, Louisiana, 10-14 July 2011.

- H.M.T. Boer, C. Stötzel, S. Röblitz, P. Deuflhard, R.F. Veerkamp, H. Woelders. A Mathematical Model of the Bovine Estrous Cycle. In: Abstract book of the 11th International Conference on Systems Biology (ICSB) PS5.4, Edinburgh, UK, 10-16 October 2010.
- H.M.T. Boer, C. Stötzel, S. Röblitz, P. Deuflhard, R.F. Veerkamp, H. Woelders. Mathematical model of the bovine estrous cycle. In: Reproduction in Domestic Animals 45(suppl. 3)p59, Proceedings of the 14th Annual Conference of the European Society for Domestic Animal Reproduction (ESDAR), Eger, Hungary, 15-18 September 2010.
- H.M.T. Boer, C. Stötzel, S. Röblitz, P. Deuflhard, R.F. Veerkamp, H. Woelders. Mathematical model of the bovine oestrous cycle. In: Proceedings of the British Society of Animal Science Annual Conference, p193, Belfast, UK, 12 - 14 April 2010.
- H.M.T. Boer, R.F. Veerkamp, H. Woelders. A systems biology approach of oestrous behaviour in dairy cows. In: Book of Abstracts of the 60th Annual Meeting of the European Association of Animal Production, p187, Barcelona, Spain, 24-27 August 2009.
- B.J. Ducro, H.M.T. Boer, T.A.E. Stout. Genetic parameters and inbreeding effects on semen quality of Friesian horses. In: Book of Abstracts of the 60th Annual Meeting of the European Association of Animal Production, p226, Barcelona, Spain, 24-27 August 2009.

Training and supervision plan

	Year
Basic package	
WIAS introduction course	2008
WGS course: Ethics and philosophy in animal sciences	2010
Scientific exposure	
WIAS Science Day, Wageningen, NL	2008-2011
Systems Biology Day, Wageningen, NL	2009-2011
5th Annual Cytoscape Public Symposium, Amsterdam, NL	2007
WIAS seminar 'strategies to improve health and fertility in dairy cows', Wageningen, NL	2008
SABRE Post-analyses workshop, Lelystad, NL	2008
4th SABRE Consortium meeting, Schiphol, NL	2009
EAAP Annual Meeting, Barcelona, Spain	2009
WIAS seminar 'Animal reproduction research at ASG-WUR and FD-UU', Wageningen, NL	2010
BSAS Annual Conference, Belfast, UK	2010
ESDAR Annual Conference, Eger, Hungary	2010
ICSB (International Conference on Systems Biology), Edinburgh, UK	2010
ADSA Joint Annual Meeting, New Orleans, USA	2011
In-depth studies	
Micro-array data analysis and meta-analysis, Edinburgh, UK	2008
ANU 30306 Nutrient dynamics (modeling exercises), Wageningen, NL	2009
Advanced visualisation, integration and biological interpretation of –omics data, Wageningen/Rotterdam, NL	2009
Introduction to mathematical modeling in biology, Wageningen, NL	2010
Professional skills support courses	
WGS course: Personal efficacy	2008
WGS course: Information literacy	2008
WGS course: Techniques for scientific writing	2009
PhD competence assessment	2009
WGS course: Project and time management	2009

WGS course: Interdisciplinary research	2010
WGS course: Career orientation	2011
Writing the general sections of a PhD thesis and its propositions	2011

Research skills training

Preparing own PhD research proposal	2008

Didactic skills training

Lecture SSB 30806 (Systems Biology)	2011

Management skills training

Organisation of WIAS Science Day	2011

Education and training total: 38 ECTS

Colophon

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