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SID 5 Research Project Final Report

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Project identification

1. Defra Project code: AC0218
2. Project title: Modelling nutritional effects on reproduction in dairy cows
3. Contractor organisation(s):
   The University of Nottingham
   School of Biosciences
   Sutton Bonington Campus
   Loughborough
   LE12 5RD
4. Total Defra project costs (agreed fixed price): £ 76,764
5. Project: start date ..... 01 October 2009
   end date ..... 31 July 2010

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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

The aim of this project was to produce a framework for modelling the bovine oestrous cycle which links nutrition, metabolism and reproduction. The reason for producing such a model is to identify and understand the key mechanisms which control reproduction in dairy cows, in particular the oestrous cycle. Being able to identify these mechanisms allows us to develop an integrated approach between nutrition and genetics to improve fertility in the UK dairy herd.

Fertility is in steady decline and represents the greatest single source of wastage in the UK dairy herd (over 50% of cows culled are removed from the herd because they are barren). Improving fertility has the added advantage of reducing the number of livestock required to meet consumer demand for milk and dairy products, thus reducing national greenhouse gas emissions. The underlying cause of poor fertility is generally acknowledged to be genetics. Although a genetic selection index for fertility has been introduced into the UK following our previous project (LK0639), it will take decades for this to have a significant impact. In other projects (LK0646, LS3306 and AC0205), we showed that nutrition has major influences on reproductive performance of dairy cows. Nutritional approaches can have immediate benefits for fertility, as confirmed by our commercial partners, but responses are not always predictable due to the complex nature of dairy cow metabolism.

To make progress, in the current project we took a systems biology approach though an interdisciplinary study involving mathematicians and animal scientists. This approach allowed us to develop a mathematical model and to identify emergent properties of the oestrous cycle. We are now able to predict numerical values for key variables within the oestrous cycle. These variables include blood concentrations of reproductive hormones (six in total), masses of follicles in the ovaries, and time of ovulation. A key finding of the project is that if these hormones do not achieve the required concentration then the oestrous cycle fails to function properly, as commonly observed in dairy cows. The advantage of using a mathematical model is that we can identify those parameters which control concentrations of hormones and thus make recommendations about which aspects of the oestrous cycle need better control. The project has also revealed several new and interesting lines of research which would help understanding of fertility in dairy cows and would allow us to tackle the increasing problem of fertility issues in the UK.

The objectives of the project were:
1) To review available mathematical models and select those meeting our requirements
2) To convert equations from selected models into standard format and units
3) To combine these equations into integrated modules and test initial model predictions against available data
4) To identify significant gaps and critical control points requiring further development.

All of these objectives have been met.

A review of existing mathematical models of the bovine oestrous cycle revealed that most had focussed only on specific aspects of the cycle, for example follicular growth, and that none modelled the cycle at a scale which permits nutrition to be incorporated easily. We therefore went back to first principles and developed a model at the whole animal scale which includes mechanisms operating in the pituitary gland and hypothalamus, located in the brain, as well as effects within the ovaries.

The model makes use of equations that represent changes in hormone concentrations within the blood and growth of follicles within the ovary, leading to ovulation. The equations realistically represent the key feedback control systems within the cow. The equations are dynamic in the sense that they constantly update themselves and make immediate responses to changes in hormone profiles. The model progressed from the simplest possible model, which reproduced the periodic nature of the oestrous cycle, to a model which includes both genetic and nutritional effects, and concentrations of other hormones. A finding of the research is that we were able to identify parameters of the model which were important in controlling the oestrous cycle and it was shown that changes in these parameters (due to either genetic or nutritional influences) would disrupt the oestrous cycle. Three important aspects of fertility in dairy cows are: resumption of normal oestrous cycles after calving; ovulation; and pregnancy establishment; the model was able to identify parameters which would influence each of these aspects.

A particularly exciting discovery was that some model parameters behave in a nonlinear fashion – in other words, they have an optimum value. For example, up to a certain breakpoint one parameter has positive effects on the number of small follicles in the ovary and size of the dominant follicle; when this parameter exceeds the breakpoint value, it has negative effects. This phenomenon was unexpected, but it fits with our experimental observations of ovarian responses to dietary manipulation of insulin in dairy cows; moving from low to moderate insulin status encourages cows to cycle, but moving from moderate to high insulin status reduces conception rate.

The project has identified a number of future lines of research that need to be explored. The model is in a form where it can be built upon and more detail added in terms of the number of variables modelled and in terms of interactions between nutrition and genetics. At present we have a model which identifies parameters that are known to be influenced by nutrition and experimental work is needed to measure exactly how these parameters are affected and to provide better parameter estimation for the model. This will enable us to predict more accurately how changes in dietary inputs lead to changes in the oestrous cycle, thereby reducing the number of abnormal cycles observed in practice. Work is needed to identify the exact mechanisms which control luteolysis of the corpus luteum and implantation of the embryo in the uterus. A major gap is that although the model predicts time of ovulation accurately, there is no prediction of the quality of the oocyte ovulated – i.e. the likelihood that pregnancy will be established. We demonstrated in previous studies that this important factor is influenced by nutrition, but there is little information in the literature on the physiological mechanisms involved.

The main outcomes of this project are:
1. Development of a new mathematical model of the bovine oestrous cycle
2. Presentation of model at a conference
3. Paper to be submitted to a peer-reviewed journal
4. A clear idea as to how to increase model complexity
5. Identification of future research needs
6. Identification of parameters which can be manipulated by diet to improve fertility

Our long-term goal is to produce fully integrated models to predict how changes in one part of the system (e.g. intake of specific nutrients) simultaneously affect other parts of the system (e.g. milk yield, insulin, follicle growth, oocyte quality, conception rate). In this way, key regulatory mechanisms and control points will be identified. This unique approach will provide biological models that can be used for research in their own right, and can be simplified to provide practical models that will be applicable to a range of on-farm scenarios within the sustainable livestock production sector. Models will aid the design of biological experiments by predicting which treatments are most likely to produce significant responses. To achieve this goal will require several years of research involving iterative modelling and biological experimentation. In the meantime, the current project constitutes a major step forward in modelling nutritional effects on reproduction in dairy cows.

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
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   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Transfer).

Introduction

Poor fertility is the major cause of wastage in the UK dairy industry and increases its environmental impact:
• 33% of cows are culled each year, after an average of only 3 lactations
• over 50% of cows culled are perfectly healthy but fail to conceive
• there is a national shortage of heifer replacements and imports pose risks for biosecurity
• the UK is no longer self-sufficient in milk and fewer crossbred animals enter the beef industry
• poor fertility adds 20% to methane and ammonia emissions from dairy herds
• projected reductions in methane emissions by 2050 will be halved if fertility is not addressed.
Improving fertility requires tools, based on sound science, which can be applied in practice.

In previous projects (LK0646, LS3306 and AC0205), we showed that nutrition influences reproductive performance of dairy cows, but the mechanisms and interactions are complex. For example, dietary stimulation of plasma insulin concentrations increased the proportion of cows that ovulated, but reduced the quality of the egg released at ovulation; however, altering insulin at strategic stages of the reproductive cycle improved pregnancy rate significantly (Garnsworthy, et al., 2009).

Further progress can come from an Integrated Systems Biology approach using mathematics to generate and test predictive models of interacting biological pathways that operate at the cell, organ, tissue, and whole-animal levels. Our long-term goal is to produce fully integrated models to predict how changes in one part of the system (e.g. intake of specific nutrients) simultaneously affect other parts of the system (e.g. milk yield, insulin, follicle growth, oocyte quality, conception rate). In this way, key regulatory mechanisms and control points will be identified. This unique approach will provide biological models that can be used for research in their own right, and can be simplified to provide practical models that will be applicable to a range of on-farm scenarios within the sustainable livestock production sector. To achieve this goal will require several years of research involving iterative modelling and biological experimentation. This 6-month project was designed as a scoping exercise to produce preliminary models based on published equations and our existing databases to provide a framework for subsequent research.

The **aim** of this six-month project was to produce a modelling framework that links nutrition, metabolism and reproduction of dairy cows in a predictive manner using dynamic, mechanistic models.

The **objectives** of the project were:
1. To review available mathematical models and select those meeting our requirements
2. To convert equations from selected models into standard format and units
3. To combine these equations into integrated modules and test initial model predictions against available data
4. To identify significant gaps and critical control points requiring further development.

All of these objectives have been met.

The **first objective**, reviewing current mathematical models and selecting those that meet our requirements, was the starting point for the project. The models reviewed included: Thompson, et al. (1969); Lacker (1981); Lacker, et al. (1987); Scaramuzzi, et al. (1993); Chavez-Ross, et al. (1997); Clement, et al. (1997); Keenan, et al. (1997); Keenan, et al. (1998); Selgrade and Sclosser (1999); Sclosser and Selgrade (2000); Soboleva, et al. (2000); Blanc and Martin (2001); Clarke, et al. (2003); Mann, et al. (2003); Soboleva, et al. (2004); Echenim, et al. (2005); Smith, et al. (2005); Reinecke, et al. (2007); Adams, et al. (2008); Kebreab, et al. (2009); Maas, et al. (2009); Smith, et al. (2009); Aerts and Bols (2010). The review process involved group meetings between mathematicians and biologists where the benefits and disadvantages of each model, in terms of the mathematics and biology modelled, were discussed. It was soon found that most of these models were too simplistic to allow nutritional effects to be included. We found that many of the models focussed on specific aspects of fertility, for example the growth of a single follicle, and thus were at the wrong scale for our requirements of including nutrition. To make progress we constructed our own model of the bovine oestrous cycle using a model of the human menstrual cycle (Selgrade and Sclosser, 1999; Sclosser and Selgrade, 2000) as starting point, but recognising the key differences between cows and humans in patterns of follicular development.

The **second objective**, converting equations from selected models into standard format and units, has been achieved by ensuring that all variables and parameters of the model have the correct SI units. This enables us to make direct comparison of numerical values produced by the model with biological data here at Nottingham or with published material. The models are coded in MATLAB, which is a well used scientific modelling package; there is further scope to convert the code to Systems Biology Markup Language (SBML) using tools developed within the Centre for Plant Integrative Biology here at Nottingham.

The **third objective**, combining equations into integrated modules and testing initial model predictions against available data, has been met by comparing hormone concentrations predicted by the model with experimental data, and we have seen that model predictions agree both qualitatively and quantitatively with experimental data. Parameter optimisation has been achieved by analysis of the model outputs with repeated simulations of the model under different circumstances. We have also identified parameters that could be described as either genetic (fixed for individual cows) or nutritional (variable) and the effects on fertility of varying these parameters has been analysed.

The **fourth objective**, identifying significant gaps and critical control points requiring further development, has been carried out throughout the project and we detail where future work is required at the end of this report.

In **summary**, the outcome of this project is that we have constructed a model framework based on sound biological principles which is able to predict hormone levels and the masses of ovarian follicles and the corpus luteum of a cow. The model is designed to allow for nutritional effects and we have started to investigate how varying the parameters of the model that are linked to nutrition can affect the ability of the cow to generate normal oestrous cycles. Further work is needed to validate and refine the model to ensure that the processes being modelled are correct. By using an integrated approach of alternating modelling with in vivo experimentation, fuller and significant progress can be made in identifying the key nutritional variables that impact on fertility in dairy cows. Much of the work of this project has been written up as a research paper which will be submitted shortly to a peer-reviewed journal.

The bovine oestrous cycle

The bovine oestrous cycle is the repeating pattern of hormonal changes which occur within the bloodstream of the whole animal and the growth of follicles and corpus luteum within the ovaries (Figure 1). The end of an oestrous cycle is marked by an ovulation and the length of a normal bovine oestrous cycle is typically 22 days (Adams, et al., 2008).

Figure 1 shows the stages of growth of ovarian follicles, which are recruitment, selection, dominance and atresia (regression of follicle) (Aerts and Bols, 2010). Our model, described in the next section, includes the same stages of growth and we are able to predict, using the model, the total mass of follicles in each stage. Follicles are recruited in a wave like pattern and as follicles grow there is competition amongst follicles to become the dominant follicle. Three waves of follicle recruitment are shown in Figure 1 and in each wave the number of follicles recruited is of the order of 10 (Ginther, et al., 2001). The number of follicles passing to subsequent stages decreases with eventually only one (dominant) follicle existing in the final state. For a three follicular wave cycle, the first two waves result in the dominant follicle undergoing atresia (death), whereas the third wave results in ovulation of the dominant follicle. Cows also commonly have two wave cycles (Ginther, et al., 1989) and the model is able to reproduce both two and three wave follicular cycles; plots of these are given in the next section.

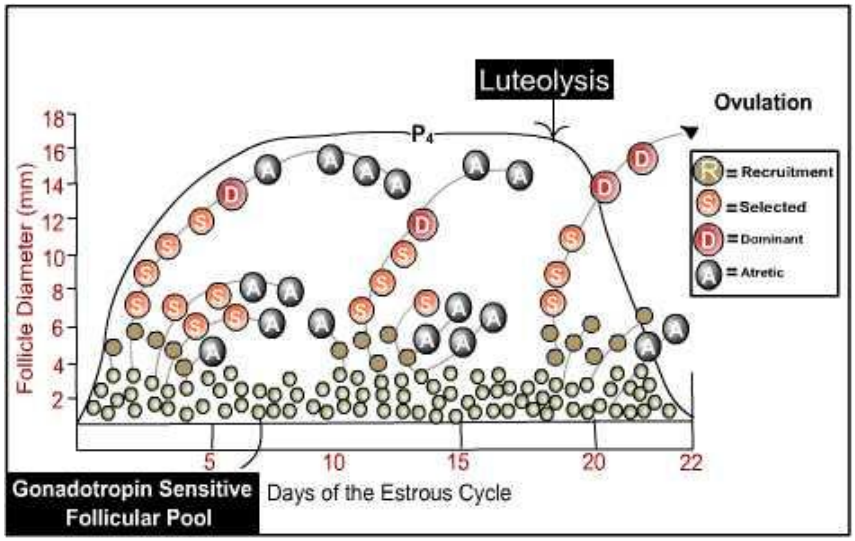

Figure 1. Sketch of an oestrous cycle with three follicular waves showing stages of growth and atresia of follicles and changes in blood progesterone (P4) concentration.

The bovine oestrous cycle is regulated by varying hormone concentrations which recruit and control the growth of follicles, as illustrated in Figure 2. Our aim is to capture and simulate those mechanisms which regulate the cycle.

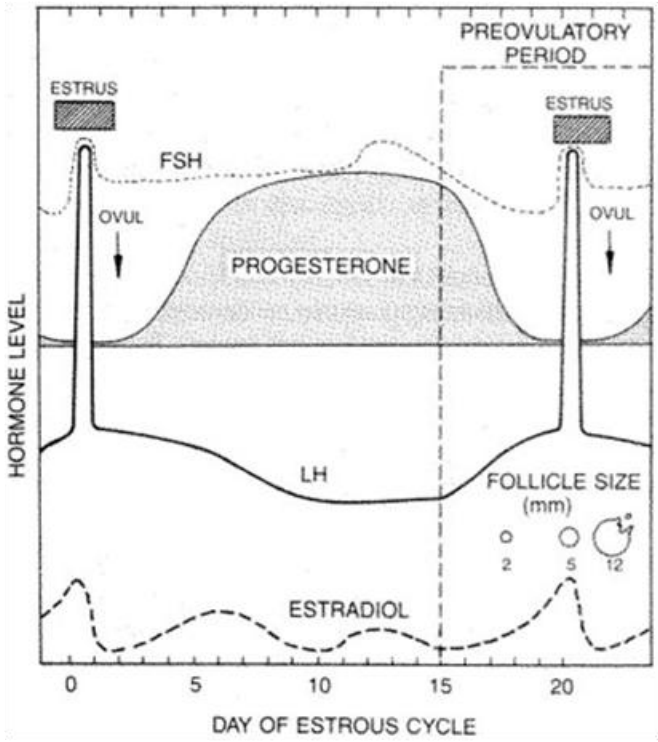

Figure 2. Changes in blood levels of follicle-stimulating hormone (FSH), progesterone, luteinizing hormone (LH) and oestradiol (ESTRADIOL) during the bovine oestrous cycle (source: extension.missouri.edu).

In the next section we use Figure 3 to help us create our mathematical model as it outlines the main interactions between hypothalamic, pituitary and ovarian hormones. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are produced in the anterior pituitary gland and released under the influence of gonadotrophin releasing hormone (GnRH) from the hypothalamus. The main hormones produced by developing follicles in the ovary are oestradiol and inhibin; these provide negative feedback to suppress release of FSH and LH by the pituitary gland.

Increasing FSH concentration stimulates recruitment of new follicles and FSH supports the growth of follicles until LH receptors are developed in the dominant stage of growth (Webb, et al., 2007). This allows the dominant follicle to survive as remaining follicles undergo atresia. Follicles produce both oestradiol and inhibin and the amount produced is assumed to be proportional to size of follicles. As follicles grow, bloodstream concentrations of oestradiol and inhibin increase which reduces FSH concentration. When a follicle becomes dominant then LH plays an important role and ovulation of the dominant follicle occurs shortly after a large surge in LH concentration

(Figure 2). There are several feedback mechanisms at work within the bovine oestrous cycle and this is what we have modelled in the next section.

As can be seen from Figures 1-3, the oestrous cycle is a complex system with concentrations of hormones controlling and regulating the various processes which occur within the ovaries, and which ultimately lead to ovulation. Being able to understand the processes and mechanisms that control the oestrous cycle is key to understanding how recommendations can be made to improve cow fertility.

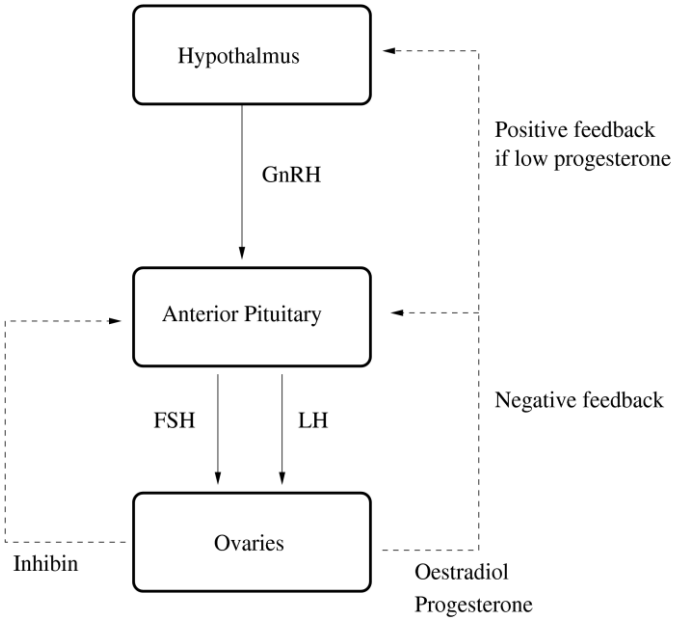

Figure 3. Major hormonal feedback mechanisms operating between the hypothalamus, anterior pituitary gland and ovaries to control the oestrous cycle (Adapted from Aerts and Bols, 2010).

Two factors that influence fertility are genetics and nutrition. Over the last few decades cows have been genetically selected for milk yield rather than for their ability to get into calf again quickly and this has had a detrimental effect on the UK's herd performance. In the short term, understanding how nutrition affects fertility and the oestrous cycle is one method where improvements in fertility can be made.

Mathematical model of the oestrous cycle

By identifying the key mechanisms which control the oestrous cycle, modelling these mathematically and solving using a numerical solver, we are able to reproduce the hormone profiles that are observed experimentally.

In Figure 4 we have represented the stages of growth of a follicle by a flow diagram with boxes showing the categories of follicles at different stages of growth. The arrows represent either transfer of follicles between categories or, if the arrow points back to the same box, growth of a follicle within that category. These processes are regulated by FSH and LH. The dashed lines represent production of oestradiol (E2) and inhibin (Ih), by the follicles.

The flow diagram in Figure 4 can be expressed as a set of differential equations which define the rate of change of total follicular mass at recruitment, selected and dominant stages of growth. The total follicular mass at each of these stages is denoted by RcF, SeF, and DmF respectively.

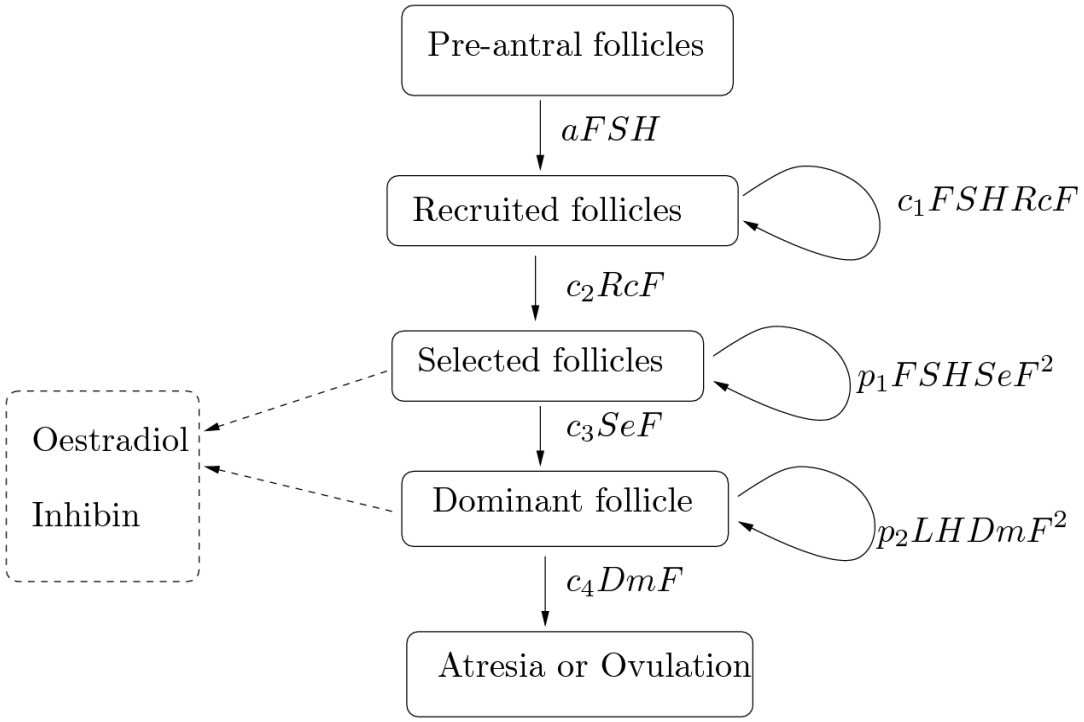

Figure 4. Flow diagram showing stages of growth of follicles and mathematical functions regulating growth of follicles within stages and transfer between stages.

The flow diagram in Figure 4 was converted into a set of differential equations which are:

$$\frac{dRcF}{dt} = aFSH + (c_1FSH - c_2)RcF$$

$$\frac{dSeF}{dt} = c_2RcF - c_3SeF + p_1FSH\ SeF^2$$

$$\frac{dDmF}{dt} = c_3SeF - c_4DmF + p_2LH\ DmF^2$$

To model oestradiol and inhibin production we used two equations where E2 represents concentration of oestradiol and Ih represents concentration of inhibin. The rate of change in concentration of these hormones can be determined by the amount of hormone released into the bloodstream and the rate at which it is cleared from the bloodstream. The rate of change of oestradiol concentration can therefore be written as:

rate of change in oestradiol or inhibin = production of oestradiol or inhibin – clearance of oestradiol or inhibin

Mathematically, this can be expressed as:

$$\frac{dE_2}{dt} = e_0 + e_1SeF + e_2DmF - \alpha_{E_2}E_2$$

Here, production of oestradiol is a function of mass of follicles in the selected stage (SeF) and dominant stage (DmF). Clearance of oestradiol is proportional to the amount of oestradiol in the bloodstream and the rate of clearance is parameterised by αE2. The rate of change of inhibin concentration, Ih, is modelled in a similar manner as:

$$\frac{dIh}{dt} = h_0 + h_1SeF + h_2DmF - \alpha_{Ih}Ih$$

We now model the feedback effects of oestradiol, inhibin and progesterone on release of GnRH, LH and FSH in Figure 3. Feedback can be either positive or negative. A positive feedback effect occurs when an increase in concentration of one hormone causes increased release of another hormone; negative feedback reduces release of a hormone. To model these mechanisms we make use of Hill functions, which are commonly used in mathematical biology. A positive feedback mechanism is modelled by the Hill function:

$$H_{+1}(x;n,T) = \frac{(x/T)^n}{1+(x/T)^n}$$

with an increase in x causing an increase in H+1. A negative feedback mechanism is modelled by the Hill function:

$$H_{-1}x;n,T = \frac{1}{1+(x/T)^n}$$

with an increase in x causing a decrease in H-1. The value of T denotes the value of x at which a switch in behaviour occurs and is commonly known as a threshold value such that a change in behaviour occurs when x is above or below T. The value of n represents how quickly this switching effect occurs, with larger values of n denoting a faster switching effect. Plots of both the positive and negative Hill functions are given in Figure 5 for various values of n, the value of T is fixed at T=5.

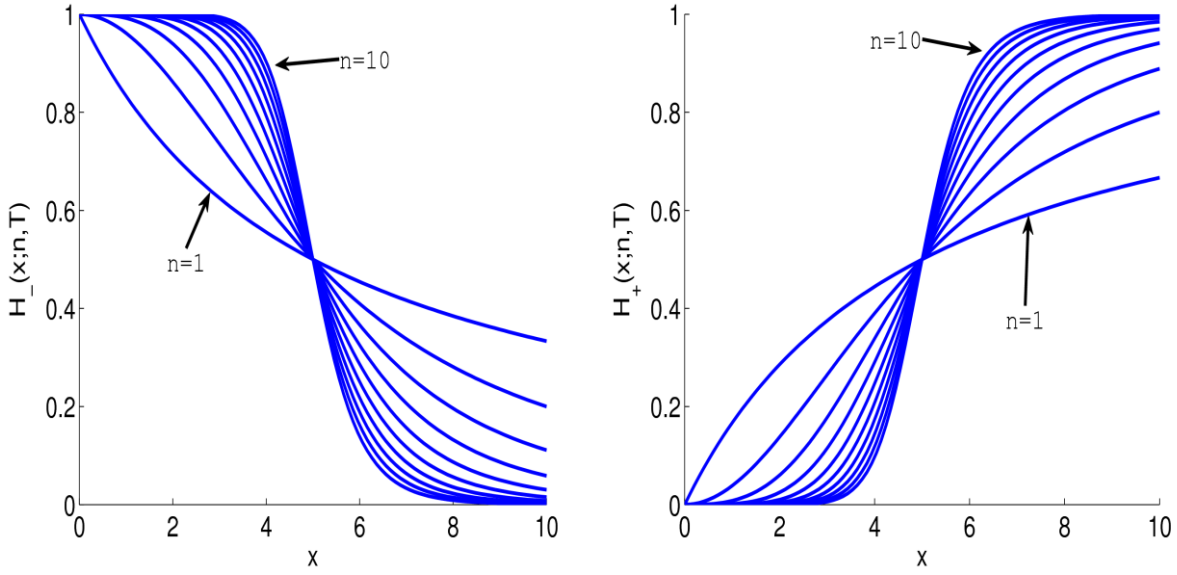

Figure 5. Plots of positive (left pane) and negative (right pane) Hill functions used to model feedback mechanisms.

The bloodstream concentration of LH is modelled as:

rate of change of LH = release of LH – clearance of LH from bloodstream

and mathematically we express this as:

$$\frac{dLH}{dt} = k_{LH}H_{+1}(GnRH;n_{LH,GnRH},T_{LH,GnRH}) - \alpha_{LH}LH$$

Here a positive Hill function has been used to model the fact that an increase in GnRH causes an increase in release of LH (Vizcarra, et al., 1997).

The equations governing concentrations of GnRH and FSH can be formed in a similar manner, but with different mechanisms controlling release of each hormone into the bloodstream. Release of GnRH is affected by concentration of progesterone (P4) and oestradiol E2; progesterone has a negative effect on release of GnRH and oestradiol has a positive effect. The equation used to describe rate of change in GnRH is:

$$\frac{dGnRH}{dt} = k_{GnRH} H_{-1}(P_4; n_{GnRH,P_4}, T_{GnRH,P_4}) H_{+1}(E_2; n_{GnRH,E_2}, T_{GnRH,E_2}) - \alpha_{GnRH} GnRH$$

Release of FSH into the bloodstream is a function of level of inhibin Ih, E2 and GnRH. Both inhibin and oestradiol have a negative effect on release of FSH; GnRH has a positive effect. The equation used to model FSH is therefore:

$$\frac{dFSH}{dt} = k_{FSH} H_{-1}(Ih; n_{FSH,Ih}, T_{FSH,Ih}) H_{-1}(E_2; n_{FSH,E_2}, T_{FSH,E_2}) + k_{FSH,GnRH} H_{+1}(GnRH; n_{FSH,GnRH}, T_{FSH,GnRH}) - \alpha_{FSH} FSH$$

We now model the corpus luteum, which completes all the essential elements of a bovine oestrous cycle. The corpus luteum is formed within in the region of the ovary which is left by the ovulating follicle of the previous cycle. The corpus luteum produces progesterone and we assume that the level of progesterone produced is proportional to mass of the corpus luteum. Figure 2 shows that progesterone concentration increases after ovulation and reaches a steady state at which concentration is constant. For a 22 day cycle, level of progesterone decreases approximately 16 days after ovulation due to luteolysis (regression) of the corpus luteum. The corpus luteum essentially goes through two stages, a growth stage from the day of ovulation until the beginning of luteolysis, and then a regression stage where the corpus luteum is undergoing luteolysis (Meier, et al., 2009).

To model the growth stage of the corpus luteum we use the following equation:

$$\frac{dCL}{dt} = r_{growth} CL \left(1 - \frac{CL}{CL_{max}}\right) for Ov_i < t < Ov_i + PGF_{release}$$

This equation is applied from time of ovulation, denoted by Ovi, until prostaglandin is released to initiate luteolysis. The number of days after ovulation at which prostaglandin is released is denoted by PGFrelease and is calculated using the following integral:

$$M = \int_{Ov_i}^{Ov_i + PGF_{release}} P_4 dt$$

Here we assume that prostaglandin is released after the uterus has been exposed to a certain amount of progesterone (Flint, et al., 1990). From the day of release of prostaglandin until ovulation, the corpus luteum undergoes luteolysis and we assume that the mass of the corpus luteum undergoes exponential decay during this time. We model this stage as:

$$\frac{dCL}{dt} = -r_{decay} CL for Ov_i + PGF_{release} < t < Ov_{i+1}$$

Rate of change in progesterone concentration (P4) is modelled as:

rate of change of concentration of progesterone = release of progesterone – clearance of progesterone

Thus, we use the following equation to model the level of progesterone:

$$\frac{dP_4}{dt} = c_{P_4} CL - \alpha_{P_4} P_4$$

This completes the model description and in the next section we show results of solving the model using numerical mathematical software.

Results

The previous section describes the equations used to model the main mechanisms controlling the bovine oestrous cycle. To test whether we included sufficient detail in our model, and to determine whether our equations do in fact model the correct behaviour, we have solved these equations using a numerical solver to determine changes in hormone concentrations and masses of follicles and the corpus luteum. In the following plots we consider predicted hormone concentrations over two oestrous cycles.

In Figure 6 we plot the hormone profiles for two oestrous cycles, predicted by the model. We see that the length of a cycle is 22 days, which agrees with what is observed experimentally (Royal et al. 2000; Adams, et al., 2008) and that three follicular waves exist, as represented by the three waves of varying concentration of FSH. We note that shortly after an increase in FSH there is an increase in oestradiol and inhibin, which correlates with an increase in follicular mass. Ovulation is denoted by the sharp rise in GnRH and LH.

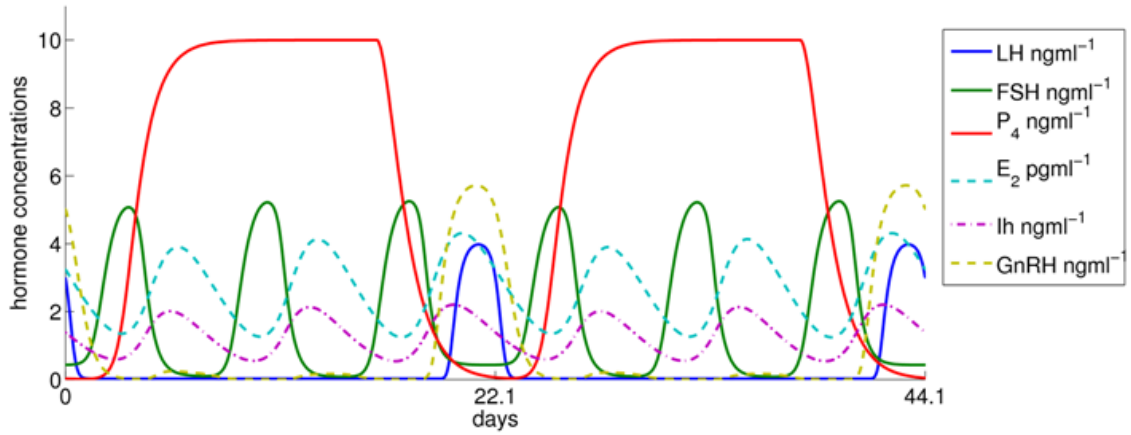

Figure 6. Profiles for luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone (P4), oestradiol (E2), inhibin (Ih) and gonadotrophin-releasing hormone (GnRH). The mass of follicles is represented by concentrations of oestradiol and inhibin and mass of the corpus luteum is related to concentration of progesterone. Days are relative to first ovulation at Day 0.

The model predicts that there will be a surge in LH and GnRH just prior to ovulation after the third follicular wave. We see a wave like pattern exists in terms of the recruitment and selection of follicles. The level of FSH changes in a three wave pattern with each peak of FSH corresponding to recruitment of follicles. Maximum concentration of FSH increases for each consecutive wave within a cycle showing that waves of follicle recruitment are not identical, something which is observed experimentally. Note that a few days after a rise in FSH the levels of oestradiol and inhibin increase and this is caused by an increase in follicular mass. The waves of FSH continue until the point at which the level of progesterone decays rapidly, allowing the level of LH to surge. This surge in LH causes ovulation to take place. If allowed to, the model would continue predicting hormone concentrations ad infinitum; here we have stopped the model after the second ovulation. Being able to let the model run for such long periods provides the opportunity to observe what happens to hormone levels over an extended time period, a requirement of being able to observe the effects of nutrition.

The model predicts hormone levels that agree with observations, which gives us confidence that the underlying biological mechanisms have been modelled in the correct manner. To test and validate the feasibility of the model, a plot of an oestrous cycle with two follicular waves is given in Figure 7. This two-wave follicular cycle was generated by changing just a few parameters in the model.

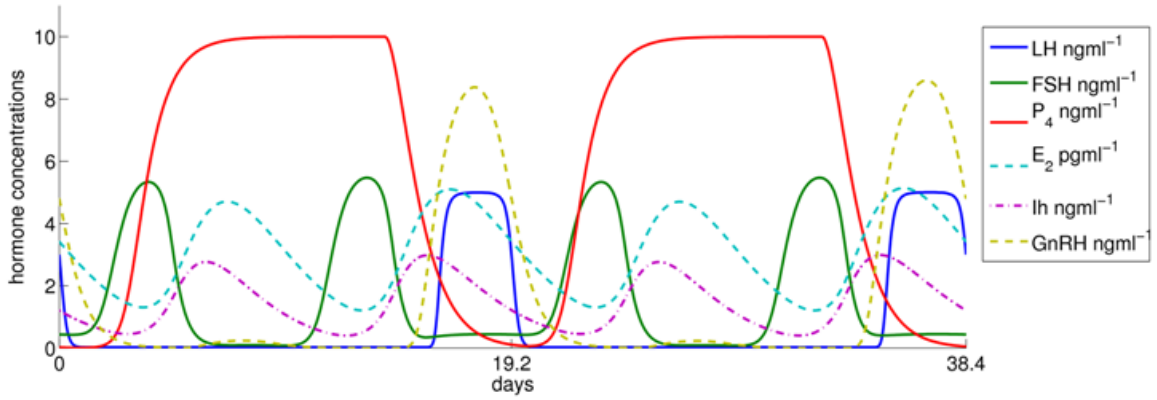

Figure 7. Hormonal profiles for a cycle with two follicular waves and 19 days between ovulations. As in Figure 6, concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone (P4), oestradiol (E2), inhibin (Ih) and gonadotrophin-releasing hormone (GnRH) are plotted.

Whether a cow has cycles with two or three follicular waves is consistent, but the biological cause is not yet fully understood. The set of parameters which alter the model from a three wave cycle to a two wave cycle suggests that rate of growth of follicles is a likely cause, and that these parameters depend on the genetics of the animal.

Several measures can be used to assess fertility of a cow and one is mass of the ovulating follicle. Constructing the model has enabled us to understand the significance of certain parameters within the model. Some parameters, for example *p1* and *p2* which control the growth of follicles, determine to a large extent the size of the dominant ovulating follicle. Figure 8 shows how varying the value of parameter *a*, which parameterizes the recruitment of follicles, has an effect on size of the dominant follicle that ovulates.

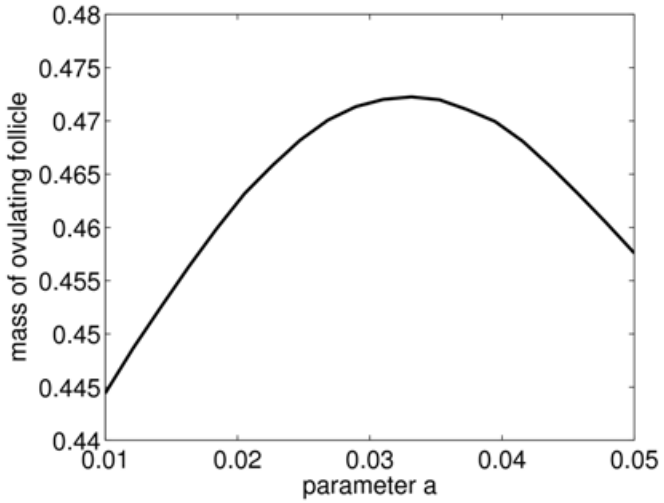

Figure 8. Effect of varying parameter a, which affects the number of follicles recruited, on size of the ovulating follicle. Note that *a* = 0.032 produces the largest follicle.

Plotting *a* against size of the dominant follicle produces a curve which is nonlinear. This means that increasing the value of *a* does not always have a positive effect. If *a* is above 0.032 then increasing *a* has a negative effect on follicle size, whereas if *a* is below 0.032 then we would want to increase *a* to achieve maximum follicle size. This outlines an effect which the model is able to predict but that experimentally might be unexpected. The model tells us that there is an optimum number of follicles to be recruited. If too few follicles are recruited, then not enough oestradiol and inhibin are produced by the follicles; if too many follicles are recruited, then too much oestradiol and inhibin are produced, inhibiting follicular growth. This could explain some of our observations in dairy cows fed on diets that induce different levels of insulin (Garnsworthy, et al., 2008a, b; 2009). In Figure 9 we plot some of our experimental data from LINK Project LK0646 showing that the number of follicles is affected by the level of starch in the diet; too much or too little starch (and hence insulin) reduces the number of follicles. This is similar to the effect of varying parameter *a* in our model.

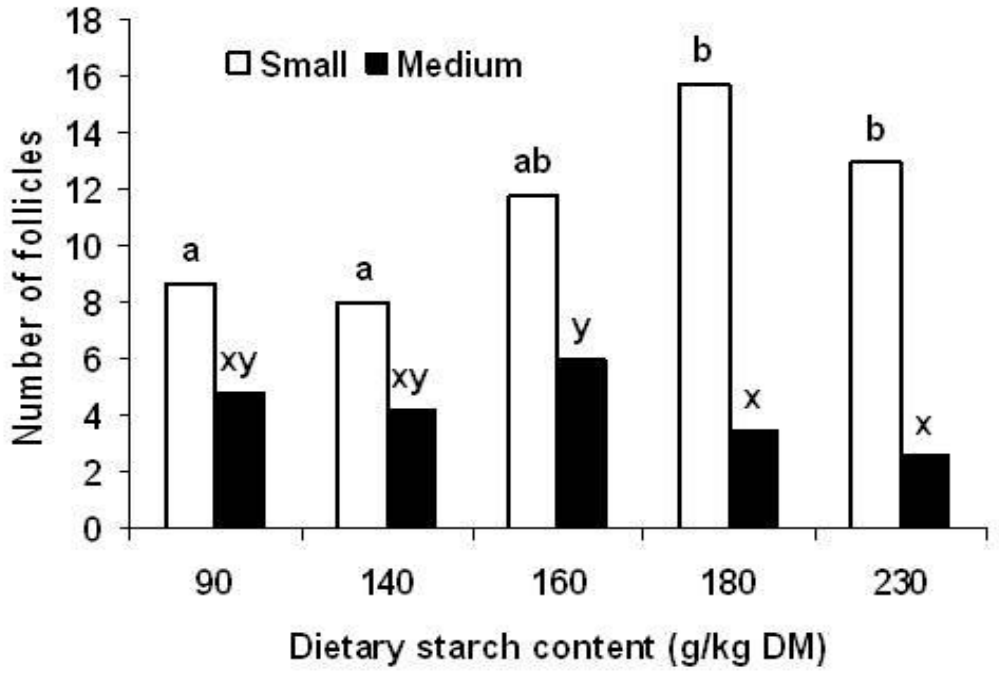

Figure 9. Effect of dietary starch content on numbers of small and medium-sized follicles (Garnsworthy, et al., 2008a).

Being able to optimise the parameters of the model is important for predicting how to improve fertility. It is not necessarily always the case that increasing a parameter has a positive (linear) effect on fertility and instead there may be nonlinear effects where an equilibrium exists, as seen in Figure 8. This is where an integrated approach between mathematics and biological experimentation can be particularly effective.

A finding of the project is that we are able to identify several parameters of the model, including parameter *a* controlling the recruitment of follicles, which are affected by the nutritional status of the animal. For example, parameters which are affected by diet are the parameters which control the growth of follicles during the different stages of growth, e.g. parameters *p1* and *p2* which regulate growth of follicles in the selected and dominant stages. Other parameters affected by nutrition include clearance rates of hormones from the bloodstream, with high planes of nutrition resulting in increased rates of clearance (Wiltbank, et al., 2007).

We are also able to identify some parameters that might be affected by genetics of the animal rather than nutrition. The parameters which control the number of follicular waves per cycle are, we believe, genetic parameters with different cows having different parameter values. These values cannot be changed by manipulating the diet of the animal. It is likely that interactions exist, so we would expect genetics to alter responses to changes in nutrition. Identifying which parameters are involved in such interactions requires further study.

Conclusions

In the six months of this project we have been able to construct a model which predicts with good accuracy the changing hormone levels of the bovine oestrous cycle. We have developed our model from biological principles based on the known feedback mechanisms of the cycle. We have demonstrated that ensuring the parameters of the model are correct is crucial in obtaining regular oestrous cycles and that varying parameter values by small amounts can disrupt the oestrous cycle. This helps to explain the difficultly in delineating key parameters and variables, and also demonstrates how regular bovine oestrous cycles can be difficult to maintain in practice if the nutritional status of a cow is suboptimal. Knowing how to control the parameters to achieve optimal fertility gives us the ability to understand better the oestrous cycle and, after appropriate validation through experiments, to give appropriate advice. We stated earlier that parameter values are linked to genetic and nutritional status and understanding how nutrition affects the parameter values is crucial in gaining greater understanding of the oestrous cycle.

Future work

In this project we have taken a systems biology approach to modelling the complexities of the oestrous cycle. Due to the inter-disciplinary nature of the work a number of questions have been raised from both a biological and mathematical viewpoint. We outline below future work which should be carried out to develop the model both mathematically and in terms of our understanding of the biology of the oestrous cycle.

Our long term aim is to produce a fully integrated model which predicts how changes in dietary intake affect fertility and milk production. This will give us the confidence to give advice on improving fertility using nutrition.

This project has produced a model which predicts changes in hormone levels of the cow and this is a good indicator of fertility since if hormone levels are abnormal then reproductive performance is impaired. By using the model we have been able to understand how varying parameters of the model affects the ability of the cow to produce regular oestrous cycles. Verifying whether these changes in parameter values produce the same effect in vivo needs further work. The optimisation of certain parameters is not always obvious and the model may be used to identify important parameters influencing the oestrous cycle.

Future work on the model could involve including many of the factors which are included in Figure 10, which illustrates the mechanisms by which nutrition affects fertility.

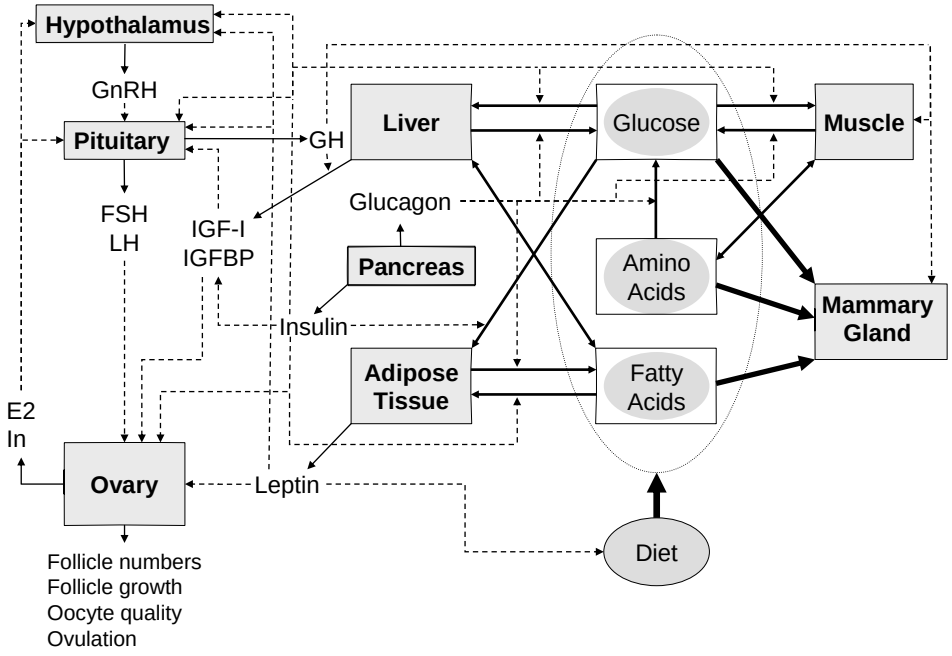

Figure 10. Interactions between diet, metabolic hormones and reproductive hormones in dairy cows (Garnsworthy, et al., 2008b).

The sophistication of the model could be increased by modelling how dietary intake affects the parameters of the model. Future work would be needed in understanding the biology of the system, but a strategy shown to improve fertility in dairy cows is a two stage approach to dietary feeding as reported in Garnsworthy, et al. (2009). This is one effect that a future model should be able to predict. However, it would require significant development work to extend the scope of the model by including nutritional effects on oocyte quality. The quality of the follicle, and in particular the oocycte contained within the follicle, is paramount to the ability of the cow to become pregnant. Another important factor is that after calving the cow will normally take 4-6 weeks to start producing healthy oestrous cycles again; for cows with low nutritional status this can take significantly longer.

The model, in terms of the mathematics, could be improved in several ways. The model could be made more biologically realistic, but mathematically more complex, by including the pulsatile nature of GnRH release from the hypothalamus. The way in which the corpus luteum has been modelled could be improved with additional hormones introduced into the model which regulate the growth/regression of the corpus luteum. However, biological understanding of the exact mechanism by which the corpus luteum undergoes luteolysis would be needed. Similarly, we have results from several in vitro studies that show how local signalling mechanisms within the ovary and the follicle affect development of follicles and oocytes.

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References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Presentation

Pring, S.R., Owen, M.R., King, J.R., Sinclair, K.D., Flint, A.P.F., Webb, R. and Garnsworthy, P.C. (2010) Modelling the bovine oestrous cycle. 42nd Meeting of the Agricultural Research Modellers' Group 26th March 2010. The Royal Society, London.
(Abstract to be published in Journal of Agricultural Science).

Journal Paper

Pring, S.R., Owen, M.R., King, J.R., Sinclair, K.D., Flint, A.P.F., Webb, R. and Garnsworthy, P.C. (2010) A mathematical model of the bovine oestrous cycle: predicting outcomes of dietary and pharmacological interventions. In Preparation.